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SOIL SCIENCE

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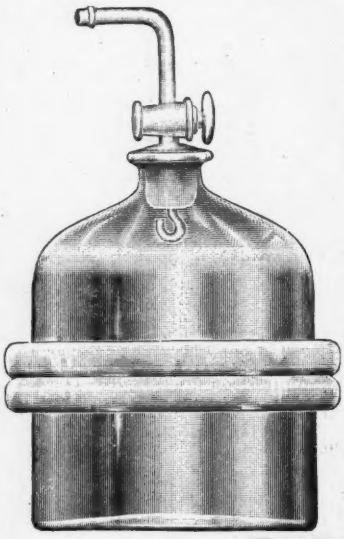

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## ERRATA

**This errata slip is to replace that issued with SOIL SCIENCE, v. 15, no. 6, opposite page 427.**

Pages 324–325. In columns 8 and 9 read $\frac{\text{Soil}}{\text{Subsoil}}$ instead of "Soil 1." Units for both columns are *per cent.*

# A 5-YEAR LYSIMETER STUDY OF THE SUPPOSED LIBERATION OF SOIL POTASSIUM BY CALCIC AND MAGNESIC ADDITIONS

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Received for publication May 21, 1923

## INTRODUCTORY

It is widely believed that burnt lime and limestone effect a liberation of potassium from the potassic components, native to the soil. As a practical application of this belief, many users of agricultural lime have assumed that the potash requirements of crops would be cared for by the supposed replacement. This assumption has been based upon the fact that solutions of neutral salts of calcium and magnesium will effect basic exchange when soil and solution are agitated or when such solutions are percolated through a mass of soil. The development of this point of view may be traced by reference to bibliographies by MacIntire (6) and by Tressler (10). However, the imposed conditions under which the active mass of the alkalis and alkali-earths may effect basic exchange are different from those which prevail in the field. There the soil is bathed not alone by neutral salts, but also by the bicarbonates of calcium and magnesium derived directly from the excess of additions, or through hydrolysis of the silicates formed where all of the larger part of the additions are absorbed by the soil.

Experimental data on the problem are meager. In 1910, Wheeler (11) reported experiments begun in 1894, wherein lime was used alone and in conjunction with sodium chloride and with potassium chloride, with results which lead to the conclusion: "The results show that liming was of no great value on the Kingston soil, as a liberator of potash . . . ." Between May 13 and September 30, 1911, Brown and MacIntire (1) subjected freshly sampled moist borings from the burnt lime plat of tier II of the Pennsylvania Station to 17 aqueous extractions. They found an average concentration of potassium less than that dissolved from the control, which indicated that after a long period of liming the native potash was either decreased in quantity or depressed in solubility. MacIntire (5) later analyzed composites of all burnt lime and limestone plats for residual potash after 30 years of cumulative liming. The results obtained by the J. Lawrence Smith method led to the statement that: "A decrease in potash was noted in each case of lime treatment, but there was no correlation between residual lime and residual potash." These analyses were made upon composite samples from four plats of $\frac{1}{8}$ acre each. The variation in soil type and topography over the experimental area and the inherent

error of sampling such large plats militate against absolute dependability upon the findings reported.

Lipman (2) questioned the liberation of potassium by $CaCO_3$ in soils as a result of his extraction studies with calcium carbonate and calcium sulfate. Lyon and Bizzell (3) found no increase in the potassium content of leachings from their 4-foot tanks containing Dunkirk soil where a 3,000-pound addition of burnt lime was made. But, since the same observation was noted where potassium sulfate was added, it appeared that the vitiating influence of the subsoil would have masked any inter-change which might have taken place. The effect of a 3,000-pound addition of burnt lime to the Volusia soil appeared to be actually depressive upon the outgo of potassium salts through the 4-foot depth. As stated by these investigators (4), "There is nothing in this experiment to indicate that the application of lime caused the liberation of potassium. The same was true of the experiment with the Dunkirk soil. It may be remarked, however, that if the application of lime did liberate any potassium from the surface soil, it may have been absorbed by the lower layers of soil and thus have been removed from the drainage water."

MacIntire (6) reported results of a 5-year lysimeter study with shallow tanks containing only surface soil and deep tanks containing both soil and subsoil, seven different calcic and magnesic materials having been added to both series. The treatments ranged from 8 to 100 tons of the several materials on constant CaO-equivalent basis. It was found that lime and magnesia additions not only failed to liberate potassium to the leachings, but that an actual depression in potassium outgo resulted. It was recognized that liberation and reabsorption was possible, but evidence was advanced to minimize the validity of such an assumption. It was concluded that depressive influences of both lime and magnesia were due to the fact that $H_2CO_3$ was a more active solvent than either $CaH_2(CO_3)_2$, or $MgH_2(CO_3)_2$, and that the amounts of $CaCO_3$ and $MgCO_3$ carried by the two bicarbonate solutions, respectively, were not in sufficient concentration to serve as liberants.

## EXPERIMENTAL

The results here offered were secured in a 5-year study of the potassium content of leachings from a loam treated with high-grade CaO and MgO, both in amounts equivalent to an application of 2,000 pounds and 3,750 pounds per 2,000,000 pounds of soil, and also with limestone and dolomite of 100-mesh fineness at the 2,000-pound-equivalent rate. The limestone contained 94.33 per cent $CaCO_3$ and 1.62 per cent $MgCO_3$, while the dolomite contained 49.96 per cent $CaCO_3$ and 39.11 per cent $MgCO_3$. In addition, both CaO and MgO were added at the 3,750-pound rate, and at the rate of 32 tons in conjunction with each of the three sulfur carriers—$FeSO_4$, pyrite, and elementary sulfur—which were used also alone as controls. Each sulfureous material was added in an amount which carried sulfur at the constant rate of 1,000 pounds of S per 2,000,000 pounds of soil. The 3,750 pound CaO-equivalents of lime

and magnesia represented a 2,000-pound addition plus 1,750 pounds to care for the immediate acidity of $FeSO_4$ and the potential acidity of the pyrite and sulfur subsequent to their sulfofication, or chemical oxidation. The influence

TABLE 1

*Potassium salts leached from "Cherokee" loam during a 5-year period—treatments of limestone and dolomite, alone and CaO, and MgO, with and without additions of $FeSO_4$, pyrite and flowers of sulfur*

| TANK NUMBER | TREATMENT | | | POTASSIUM PER 2,000,000 LBS. OF SOIL | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | CALCIC-MAGNESIC | | Sulfur constant of 1,000 lbs. per 2,000,000 lbs. of soil | Annual period | | | | | 5-year period | | |
| | Material | CaO equivalent, per 2,000,000 lbs. of soil | | First | Second | Third | Fourth | Fifth | Average annual | Total | Increase or decrease over no-treatment control |
| | | | | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* |
| 50 | None | None | None | 11.8 | 8.4 | 12.7 | 7.0 | 6.2 | 9.2 | 46.1 | ..... |
| 51 | Limestone | 2,000 lbs. | None | 9.0 | 7.0 | 9.0 | 9.5 | 7.8 | 8.5 | 42.3 | −3.8 |
| 52 | Dolomite | 2,000 lbs. | None | 7.4 | 5.5 | 9.6 | 8.5 | 4.1 | 7.0 | 35.1 | −11.0 |
| 53 | CaO | 2,000 lbs. | None | 9.2 | 6.7 | 10.2 | 11.2 | 4.7 | 8.4 | 42.0 | −4.1 |
| 54 | CaO | 3,750 lbs. | None | 5.6 | 6.8 | 11.6 | 7.5 | 4.1 | 7.1 | 35.6 | −10.5 |
| 55 | MgO | 2,000 lbs. | None | 7.7 | 6.1 | 8.5 | 5.1 | 4.4 | 6.4 | 31.8 | −14.3 |
| 56 | MgO | 3,750 lbs. | None | 8.5 | 6.3 | 9.8 | 8.9 | 4.2 | 7.7 | 37.7 | −8.4 |
| 57 | None | None | $FeSO_4$ | 14.8 | 10.2 | 9.6 | 12.2 | 6.6 | 10.7 | 53.4 | 7.3 |
| 58 | CaO | 3,750 lbs. | $FeSO_4$ | 6.0 | 7.3 | 10.2 | 7.7 | 4.5 | 7.1 | 35.7 | −10.4 |
| 59 | MgO | 3,750 lbs. | $FeSO_4$ | 5.3 | 5.7 | 15.4 | 8.3 | 3.3 | 7.6 | 38.0 | −8.1 |
| 60 | CaO | 32 tons | $FeSO_4$ | 5.4 | 5.3 | 14.6 | 5.5 | 4.9 | 7.1 | 35.7 | −10.4 |
| 61 | MgO | 32 tons | $FeSO_4$ | 10.3 | 4.4 | 6.9 | 4.2 | 2.3 | 5.6 | 28.1 | −18.0 |
| 62 | None | None | Pyrite | 11.6 | 10.6 | 11.3 | 10.8 | 6.6 | 10.2 | 50.9 | 4.8 |
| 63 | CaO | 3,750 lbs. | Pyrite | 6.5 | 10.8 | 8.1 | 7.5 | 5.2 | 7.6 | 38.1 | −8.0 |
| 64 | MgO | 3,750 lbs. | Pyrite | 8.7 | 6.0 | 9.5 | 3.5 | 2.5 | 6.0 | 30.2 | −15.9 |
| 65 | CaO | 32 tons | Pyrite | 6.4 | 7.0 | 9.6 | 8.8 | 5.1 | 7.4 | 36.9 | −9.2 |
| 66 | MgO | 32 tons | Pyrite | 8.2 | 4.6 | 7.6 | 4.5 | 2.4 | 5.5 | 27.3 | −18.8 |
| 67 | None | None | Sulfur | 15.1 | 11.1 | 14.7 | 9.4 | 5.3 | 11.1 | 55.6 | 9.5 |
| 68 | CaO | 3,750 lbs. | Sulfur | 6.6 | 10.7 | 10.4 | 8.9 | 5.2 | 8.4 | 41.8 | −4.3 |
| 69 | MgO | 3,750 lbs. | Sulfur | 12.7 | 7.7 | 8.6 | 7.6 | 3.5 | 8.0 | 40.1 | −6.0 |
| 70 | CaO | 32 tons | Sulfur | 8.4 | 6.5 | 7.7 | 11.0 | 6.0 | 7.9 | 39.6 | −6.5 |
| 71 | Mgo | 32 tons | Sulfur | 8.0 | 3.5 | 7.4 | 3.6 | 2.3 | 5.0 | 24.8 | −21.3 |

of CaO and MgO upon the chemical and bacterial oxidative processes responsible for the formation of sulfates is reported in another contribution (8) now in the hands of the printer.

All treatments were mixed uniformly throughout the mass of soil. The soil used was secured from a near-by source and was protected to prevent drying after mixing and screening, and during the overnight determination of moisture. The soils were mixed and placed in 1/20,000-acre galvanized "ingot" iron, asphaltum-coated tanks, August 3, 1917, since which time they have been subjected to prevailing weather conditions. Each tank contains a sand filter-bed, from which runs a block-tin tube drainage outlet. This outlet passes through a reinforced concrete retaining wall and leads into the asphaltum-coated galvanized iron receptacles (6). As made necessary by distribution of rainfall, the leachings are collected, conveyed to the laboratory and after certain analyses on the separates are retained as aliquot-composites, acidified with HCl. At the end of each annual period the composites are analyzed for potassium by the colorimetric method of Schreiner and Failyer (9). The annual and total outgo of potassium from each of the 22 tanks for the years 1917 to 1922 are given in table 1.

## DISCUSSION OF RESULTS

The amounts of potassium removed from the soil by leachings from natural rainfall were small. Nevertheless, certain consistent differences are shown. The results will be considered, however, on the basis of the total loss, with the detail of the annual losses left to the inspection of the interested student. However, the entire amount of leached potassium salts may not have been derived from the soil. Some potassium sulfate is precipitated in the rain-water at this point, as has been shown by MacIntire, Shaw and Young (7); but, this increment is a constant and will be considered as though native to the soil.

The total losses from both limestone and dolomite were less than the outgo from the control. The same was true also of the burnt lime and magnesia at both 2,000-pound and 3,750-pound rates. The two magnesia treatments show an average depression greater than that of the two lime additions. This is in harmony with previous findings (6) secured by the use of another soil.

The $FeSO_4$ group shows a decrease in outgo where the four lime and magnesia additions were made and an increase where $FeSO_4$ was added without a basic supplement. The augmented outgo of calcium and magnesium sulfates from the $FeSO_4$ acid-soil control and the absence of any $FeSO_4$ in the leachings demonstrated that the sulfate treatment was practically the same, primarily, as an equivalent treatment of sulfuric acid and, secondarily, as an equivalent of a mixture of $CaSO_4$ and $MgSO_4$. Such a treatment would be expected to effect the liberation of potassic salts. But, if the leached potassium may be taken as a quantitative measure of the liberation effected, the 1,000 pound sulfate-sulfur addition, equivalent to 3,747 pounds of $CaSO_4$, has liberated only 7.3 pounds of potassium, or a replacement ratio of 1:0.00195, as representing the full effect over the 5-year period. However, only 934.6 pounds of sulfate sulfur, or 719.9 pounds in excess of that in the rainfall, has been recovered

during the full 5-year period, 534.3 pounds having been leached during the first year. Practically the full recovery of the sulfate radical was obtained during the first year, where the heavy MgO supplement was incorporated and recoveries of 82.9 per cent and 92.4 per cent were obtained from the 3,750-pound CaO and MgO additions, respectively. Thus it appears that the liberative action of the calcium and magnesium sulfates evidenced in the $FeSO_4$ acid soil control was either prevented or repressed by a secondary action of the added excess of the two alkali-earths. In other words, the more concentrated solutions of soil-derived sulfates with a minimum of calcium and magnesium bicarbonates gave an increased outgo of potassium salts; but, solutions of even greater concentration of calcium and magnesium sulfates with an increase in respective bicarbonates proved depressive to potassium outgo. As applied to two of our residual soils, this constitutes cumulative evidence to the effect that the *bicarbonates of calcium and magnesium are depressive rather than inactive in their influence upon the outgo of potassium.* It may be that the two alkali-earths are responsible for the formation of hydrated oxides of iron, and possibly of aluminum, and that through adsorption these two products, retard the potassium outgo in the free soil-water leachings.

In another contribution now in print (8), we have shown that the oxidation of pyrite, as evidenced by sulfate outgo, was more rapid in the control than in the four tanks to which lime or magnesia was added. The pyrite treatment therefore represents a progressive ferrous sulfate addition. In like manner, in corresponding restricted measure, the four CaO and MgO additions with pyrite represent treatments of $CaSO_4$ and $MgSO_4$, respectively, with the bicarbonates in larger quantities, and these show a distinct retardative effect upon potassium outgo. In this series, as was true also of the $FeSO_4$, and elementary sulfur group, the heavy MgO treatment proved the most depressive of the five conditions.

In the contribution above mentioned, it is shown that the sulfate outgo from the unsupplemented addition of sulfur was almost as rapid as that from the $FeSO_4$ control and it is therefore not surprising to find that the increase in potassium outgo from the sulfur alone was as great as that from the added sulfate. As differing from the pyrite group, however, the light lime and both light and heavy magnesia additions accelerated the generation and outgo of sulfates from the additions of sulfur. Nevertheless, in spite of the more rapid and greater ultimate outgo of calcium and magnesium sulfates from the specified treatments of this series, the excess of bicarbonates again proved to be depressive to the sulfate outgo, as was the case in the $FeSO_4$ parallel.

These findings, as a whole, demonstrate that calcic and magnesic oxides are depressive to potassium outgo when the two materials are used in economic and practical amounts, as well as in excess, and that the same proves true of 100-mesh limestone and dolomite in chemical equivalence and at the rate ordinarily used. The results do not prove, however, that the alkali-earth treatments would serve as deterrents to the assimilation of potassium by

plants of such extensive root development as the clovers. For, the basic treatments activate the flora which generate nitrates and sulfates and the enhanced supplies of these nutrients and the development of a favorable soil reaction would so stimulate the young plants as to cause a spread of their root growth, thus increasing their ability to forage for the needed potassium.

Calcium and magnesium losses are not given. It was found, however, that soil-derived calcium and magnesium sulfates appeared coincidently with increases in potassium leachings from the unlimed tanks. It is not proved that this result is due to the liberation of potassium through basic interchange.

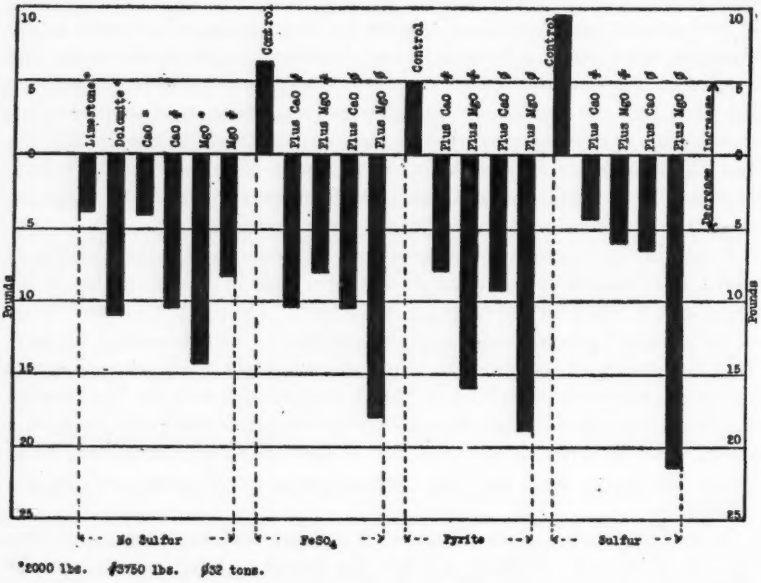


FIG. 1. INCREASE OR DECREASE IN 5-YEAR POTASSIUM OUTGO AS CAUSED BY CALCIC AND MAGNESIC TREATMENTS OF CHEMICAL EQUIVALENCE

Variation from base line shows pounds of potassium per 2,000,000 pounds of soil.

But, assuming that the small increases in potassium outgo from the non-basic treatments were due to basic interchange, and that there was no extensive readsorption by the soil, the increase in soluble potassium would represent only a fraction of the amount which would ordinarily be applied as a fertilizer.

## SUMMARY

A study of the leaching of potassium from a 23-unit lysimeter equipment during a 5-year period is reported in this paper. The influence of calcic and magnesic materials upon the liberation of potassium from the soil was deter-

mined by additions of limestone, dolomite, CaO and MgO in chemical equivalence, at the rate of 2,000 pounds of CaO per 2,000,000 pounds of soil. Both CaO and MgO were used also at an equivalent rate of 3,750 pounds. Five treatments each of $FeSO_4$, pyrite, and elementary sulfur also were used, four of each set receiving supplements of lime or magnesia each at two rates.

The results show that every form and rate of calcic and magnesic treatment, alone and in conjunction with sulfates, was depressive to the outgo of potassium Increases over the control were obtained in the three cases of sulfur carriers when unsupplemented by either alkali-earth, but such increases were found to represent only a minute fraction of the theoretical liberation to be accredited to the amounts of calcium and magnesium sulfates derived from the sulfureous treatments.

## REFERENCES[1]

(1) BROWN, B. E., AND MACINTIRE, W. H. 1911 The relation of certain water-soluble soil constituents in plots 16–14. *In* Penn. State Col. Ann. Rpt. 1910–11, p. 105.

(2) LIPMAN, C. B., AND GERICKE, W. F. 1918 Does $CaCO_3$ or $CaSO_4$ affect the solubility of the soil's constituents? Univ. Cal. Pub. Agr. Sci., v. 3, no. 10, p. 271.

(3) LYON, T. L., AND BIZELL, J. A. 1918 Lysimeter Experiments: I. N. Y. (Cornell) Agr. Exp. Sta. Mem. 12, p. 7–115.

(4) LYON, T. L., AND BIZZELL, J. A. 1921 Lysimeter Experiments: II. N.Y. (Cornell) Agr. Exp. Sta. Mem. 41, p. 71.

(5) MACINTIRE, W. H. 1912 Results of thirty years of liming. *In* Penn. State Col. Ann. Rpt. 1911–12, p. 73.

(6) MACINTIRE, W. H. 1919 The liberation of soil potassium induced by different calcic and magnesic materials, as measured by lysimeter leachings. *In* Soil Sci., v. no. 5, p. 337–397.

(7) MACINTIRE, W. H., AND YOUNG, J. B. 1923 Sulfur, calcium, magnesium and potassium content and reaction of rainfall at 10 points in Tennessee. *In* Soil Sci., v. 15, no. 3, p. 205–227.

(8) MACINTIRE, W. H., SHAW, W. M., AND YOUNG, J. B. 1923 Influence of calcic and magnesic treatments upon sulfate leachings from soil alone and with additions of ferrous sulfate, pyrite and sulfur. *In* Soil Sci., v. 16, p. 159–182.

(9) SCHREINER, O., AND FAILYER, G. H. 1906 Colorimetric, titration, and turbidity methods. U. S. Dept. Agr. Bur. Soils Bul. 31, p. 31–33.

(10) TRESSLER, D. K. 1918 The solubility of soil potash in various salt solutions. *In* Soil Sci., v. 6, no. 3, p. 237–257.

(11) WHEELER, H. J. 1910 Studies of the needs of Rhode Island soils. *In* R. I. Agr. Exp. Sta. Bul. 139, p. 75.

[1] Since preparation of this manuscript, a related article has been contributed by D. E. Haley, *In* Soil Sci., v. 15, no. 3, p. 167.

# THE ABSORPTION OF IONS BY PLANTS

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## INTRODUCTION

The absorption of ions from the soil solution and their functions in the plant must always be regarded as among the most important problems of soil and plant interrelations. It is not sufficient to study only the soil and its dissolved components; the plant must likewise receive consideration, if we are to understand the whole chemical system involved in plant growth. It is essential to such an understanding to learn something of the processes by which ions are absorbed from culture media. To what extent does one ion influence the absorption of another ion? How is absorption affected by the reaction and by the concentration of the solution? What is the relation between the intake of solutes and of water? These questions illustrate the nature of the discussion upon which we are now entering.

Considerable attention has previously been given in this laboratory to the question of the absorption of inorganic solutes by plants at different stages of growth. The methods of soil, sand and solution cultures have all been employed at various times. These experiments have been continued under simplified conditions for the purpose of obtaining data bearing more directly on the relative absorption of different ions, and on related questions.

The course of absorption must generally be followed with the aid of chemical analysis applied either to the culture solution or to the plant tissues, and the data so obtained do not necessarily represent degrees of cell permeability, as has been pointed out by Osterhout (12) and by Brooks (1). The determination by chemical methods of the intake of ions by higher plants, it will readily be granted, does not always offer the most favorable means of approach to the study of the chemistry of the plant cell. It appears essential, nevertheless, to carry out such investigations from the point of view of the relations existing between a plant and its culture medium. Otherwise we have no basis for the interpretation of many experiments bearing on important problems of plant nutrition. Meanwhile, it is confidently to be expected that researches of the type recently reported by Loeb (10), Osterhout (14), and other physiologists, will eventually enable us to overcome some of the difficulties now constantly confronting the investigator of plant metabolism.

[1] The writer desires to acknowledge the invaluable assistance of Mr. J. C. Martin, Instructor in Plant Nutrition, in connection with a portion of the analytical work.

A considerable number of experiments dealing with the absorption of essential and non-essential elements by plants have been described, among which may be noted those of Pantanelli (15), Waynick (20), and recently, of Redfern (16). The general literature has been discussed by these investigators and no attempt will be made in this article to review the many citations. It does not appear, however, that the investigations so far reported have been definitely planned to answer certain questions the discussion of which forms the main purpose of the present paper. In many of the previous investigations, the analytical control was incomplete, and the number of plants used too small. The importance of hydrogen-ion concentration was, of course, not known to the earlier workers.

Before discussing the data pertaining to the removal of ions from solution, it is desirable to make a statement dealing with the changes of hydrogen-ion concentration produced by the growth of plants. It was shown in earlier work that complete culture solutions containing nitrate tended to have their reaction changed in the direction of the neutral point. Under the experimental conditions then adopted, it was also observed that no considerable increase of acidity occurred when plants were grown in single salt solutions. Later and more extensive experiments have shown that the growth of barley and pea seedlings may produce in the solutions of certain salts, e.g., $K_2SO_4$ and $(NH_4)_2SO_4$, an intensity of acidity which may be accompanied by injury to the plant, after sufficient lapse of time. This phase of the work is discussed in another article in more detail, but the pH values of the solutions used in these experiments are included in a number of the tables.

The object of the first series of experiments was to determine the effect of a positively charged ion on the absorption of an ion of opposite charge, and vice versa. Numerous series of solutions were used, in some cases keeping the cation the same and in others the anion. Equivalent concentrations of salts were chosen so that in each set of cultures, the common ion would be present in the same concentration in all the solutions. The magnitudes of the total concentrations were similar to those found in ordinary culture solutions and in soil solutions.

## EXPERIMENTAL TECHNIQUE

In each experiment the plants were first grown during a preliminary period in a complete culture solution and then the entire group of cultures was divided into uniform sets of either 49 or 98 plants. The tumblers which were used as culture vessels had a capacity of 120 cc. and seven plants were placed in each tumbler. Before transferring the plants to the new solutions, the roots were thoroughly rinsed with distilled water. The periods over which the absorption of ions was measured were, in most cases, limited to 2 or 3 days. At the end of these periods, the plants were removed and the residual solutions were analyzed. The tops of the plants from each set of cultures were weighed green, and in some instances the dry weights of tops and of roots also were recorded. Barley, of the Beldi variety, or Four Thousand, was used in all experiments. The time allowed for absorption from the special solutions was made brief, since if plants are grown for longer periods in different solutions, pronounced variations in their size and stage of development may occur and these variations

may complicate any interpretation of the results. It is obvious, also, that single salts or simple mixtures of salts cannot serve for extended periods of growth. Our intention was to attempt a comparison of the relative absorption of ions from different solutions by plant systems as nearly alike as possible.

In the majority of the experiments, distilled water was added to the cultures at intervals so as to maintain the volumes of solution approximately constant; in other experiments, no water was added to make up for loss by transpiration, and the solutions were analyzed without dilution. This technique was adopted when it was desired to compare the relative absorption of water and of ions.

Care was taken to prevent the creeping of solution up to the cotton which held the plants in place in the cork. By the time the plants were ready for study, the stems usually almost completely filled the holes in the corks, so that the cotton could be removed, otherwise new pieces of cotton were inserted and kept dry.

The plants were grown on a balcony with full exposure to sunlight, at different seasons of the year. The preliminary periods referred to above were generally about three weeks in length, but varied according to weather conditions. The complete culture solution in which the plants were started was similar to that used for many years in this laboratory and which has been found to permit of excellent development of many types of plants. Ordinarily, only two portions of this solution were supplied, since it was the intention to prevent, as far as possible, any excess absorption of ions during the first period. In all cases, the plants had a very thrifty appearance, and possessed excellent root systems.

The amounts of ions removed from the solutions were determined by chemical analysis rather than by conductivity measurements. While the latter method is far less laborious, and has yielded results of interest in the hands of True and Bartlett (19) it is obviously not capable of a definite interpretation in terms of individual ions or chemical elements which is one of the most interesting aspects of the question. The analytical methods were those long employed in this laboratory for the examination of soil extracts and culture solutions. Complete analyses of many of the solutions made it possible to take into consideration, not only the ions originally present, but those given off by the plant roots, whether as a result of leaching from dead cells, or of exchange of ions in the cell wall or protoplasm. Complete analyses also possess the advantage that equivalents of positive and negative ions may be balanced and the accuracy of the work checked. While such complete analytical data were obtained in many experiments, in order to conserve space, only the most significant figures are given here. All the values have been calculated in terms of milli-equivalents (reaction values).

Whenever a complete analysis was made and the positive and negative ions values compared, it was found that the agreement was within the limits of error generally recognized as permissible in the analysis of solutions of this type.

The comparatively large number of plants used lessened any errors of variability. No importance is attached to any but very considerable differences in the quantities of ions absorbed. Frequently duplicate experiments were made and the results agreed in all instances within limits of much smaller magnitude than those considered as significant for our present purposes. Since determinations of the absorption of ions by individual plants in the large number experimented with could not be made, the data for absorption are, of course, not amenable to statistical treatment. In two typical experiments the dry weights of individual cultures (each made up of 7 plants) were obtained and the probable error of the mean calculated. This was found to be about 6 per cent, which is similar to the values found by Davis (4) for various sets of wheat plants grown in different culture solutions. Assuming that the variability in absorption of ions by individual plants is of the same order of magnitude as that of dry weights, the differences considered significant in the present paper are greater than those assignable to variability. For these various reasons, it is justifiable to believe that the factor of variability does not enter into the conclusions which are drawn.

With regard to the possible toxicity of single salt solutions, it may be noted that the plants showed no evidence of injury, and this would scarcely be expected, considering the brief duration of the contact between the plants and the solutions, and the low concentration of the latter. Moreover, after barley plants have been growing in a complete culture solution for some time, they may later continue to grow in single salt solutions for considerable periods without injury. Injurious hydrogen-ion concentrations were not developed under the experimental conditions.

### ABSORPTION OF POTASSIUM FROM DIFFERENT SALTS

Turning now to a consideration of the data presented in table 1, we find marked variations in the amounts of potassium absorbed which are dependent upon the anion in equilibrium with the potassium ion. The greatest absorption occurred from the solution of potassium nitrate, the least absorption from the solution of potassium sulfate. Potassium was absorbed to a decidedly greater extent from the chloride than from the sulfate solution. In this experiment potassium and chlorine ions were absorbed in practically equivalent quantities. No such equivalence of absorption was found with the other salts. Potassium ions were removed from solution in greater equivalent quantity than sulfate, phosphate, or bicarbonate ions, while the absorption of nitrate ions was nearly double that of potassium ions, the former being replaced in the solution by bicarbonate ions. In the case of potassium sulfate, the solution became slightly more acid, and also calcium and other ions, derived from the roots, replaced potassium to a certain extent.

### ABSORPTION OF CALCIUM AND MAGNESIUM FROM DIFFERENT SALTS

The experiments carried out with calcium salts (table 2) gave evidence of approximately equivalent absorption of cations and of anions from solutions of either calcium sulfate or calcium phosphate. From the chloride and nitrate solutions, the anion was removed in significantly larger quantity than the cation. The equivalents of nitrate removed were about six times those of calcium. It is very interesting to observe that chlorine ions, as well as nitrate ions could be replaced in the solution by bicarbonate ions.

Two salts of magnesium were studied, nitrate and sulfate (table 3). The nitrate was absorbed in greater equivalent amounts than magnesium, while sulfate was removed from solution to a lesser extent than magnesium. In each case, there was a tendency for the hydrogen-ion concentration to decrease. The magnesium nitrate solution became almost neutral.

### ABSORPTION OF NITRATE FROM DIFFERENT SALTS

In another series of cultures, four different nitrate solutions of the same concentration of $NO_3$ were compared (table 4). In every case, $NO_3$ ions were removed to a much greater extent than the respective cations. The reactions

of the solutions were changed to approximate neutrality. The disproportion between the absorption of anions and cations is greater with calcium and magnesium nitrates than with sodium and potassium nitrates.

TABLE 1

*Absorptions of ions from solutions of different potassium salts*

| NAME OF SALT | INITIAL CONCENTRATION | IONS ABSORBED BY PLANT | | IONS ENTERING SOLUTION | | | AGE OF PLANTS | LENGTH OF ABSORPTION PERIOD |
|---|---|---|---|---|---|---|---|---|
| | | K | Anion | Ca | Mg | $HCO_3$ | | |
| | *atm.* | *milli-equivalents* | | | | | *days* | *days* |
| *Experiment 1A* | | | | | | | | |
| $K_2SO_4$ | 6.60 | 1.44 | | 0.50 | 0.53 | | 28 | 2 |
| | 6.60 | 1.57 | | 0.50 | 0.49 | | | |
| KCl | 6.48 | 2.67 | 2.40 | 0.38 | 0.44 | 0.68 | | |
| | 6.48 | 2.57 | 2.51 | 0.47 | 0.45 | 0.82 | | |
| *Experiment 2* | | | | | | | | |
| $K_2SO_4$ | 6.32 | 0.74 | 0.40 | 0.32 | 0.24 | | 25 | $1\frac{1}{2}$ |
| KCl | 6.32 | 1.28 | 1.30 | 0.24 | 0.18 | 0.36 | | |
| $KNO_3$ | 6.45 | 1.66 | 3.19 | 0.19 | 0.21 | 1.89 | | |
| $KH_2PO_4$ | 6.32 | 0.90 | 0.14 | 0.28 | 0.21 | | | |
| $KHCO_3$ | 6.30 | 1.20 | 0.84 | 0.24 | 0.20 | | | |

| NAME OF SALT | INITIAL CONCENTRATION | K ION ABSORBED BY PLANT | REACTION | | $H_2O$ ABSORBED | AGE OF PLANTS | LENGTH OF ABSORPTION PER OD |
|---|---|---|---|---|---|---|---|
| | | | Initial | Final | | | |
| | *atm.* | *milli-equivalents* | *pH* | *pH* | *cc.* | *days* | *days* |
| *Experiment 3* | | | | | | | |
| KCl and $CaCO_3$ suspension | 6.22 | 1.74 | 6.3 | 6.3 | 550 | 35 | 2 |
| $K_2SO_4$ and $CaCO_3$ suspension | 6.22 | 1.12 | 6.3 | 5.8 | 540 | | |
| *Experiment 4* | | | | | | | |
| KCl and $CaCO_3$ suspension | 6.20 | 0.91 | 6.9 | 6.8 | 610 | 19 | 1 |
| $K_2SO_4$ and $CaCO_3$ suspension | 6.22 | −0.02 | 6.9 | 6.8 | 670 | | |

TABLE 2

*Absorption of ions from solutions of different calcium salts*

| NAME OF SALT | INITIAL CONCENTRATION | IONS OBSERVED BY PLANT | | IONS ENTERING SOLUTION | | | REACTION | | $H_2O$ ABSORBED | AGE OF PLANTS | LENGTH OF ABSORPTION PERIOD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ca | Anion | K | Mg | $HCO_3$ | Initial | Final | | | |
| | *atm.* | *milli-equivalents* | | | | | *pH* | *pH* | *cc.* | *days* | *days* |
| *Experiment 1* | | | | | | | | | | | |
| $CaCl_2$ | 4.90 | 0.73 | | 0.13 | 0.48 | | | | | 21 | 3 |
| $CaCl_2$ | 4.90 | 0.83 | 2.06 | 0.11 | 0.34 | 1.26 | | | | | |
| $Ca(NO_3)_2$ | 4.90 | 1.21 | 4.50 | 0.07 | 0.38 | 3.44 | | | | | |
| $Ca(NO_3)_2$ | 4.90 | 1.25 | 4.54 | 0.12 | 0.44 | 3.69 | | | | | |
| *Experiment 2* | | | | | | | | | | | |
| $CaCl_2$ | 12.50 | 1.50 | 2.37 | 0.02 | 0.28 | 1.12 | 5.8 | 6.6 | 625 | 21 | 2 |
| $Ca(NO_3)_2$ | 12.70 | 1.66 | 5.48 | 0.09 | 0.20 | 4.12 | 5.6 | 7.0 | 510 | | |
| $Ca(H_2PO_4)_2$ | 12.30 | 1.03 | 0.95 | 0.20 | 0.27 | | 4.2 | 4.2 | 590 | | |
| $CaSO_4$ | 12.30 | 0.88 | 0.83 | 0.09 | 0.20 | 0.32 | 6.4 | 6.6 | 715 | | |
| *Experiment 3* | | | | | | | | | | | |
| $CaSO_4$ | 4.59 | 0.00 | 0.00 | 0.04 | 0.16 | 0.47 | 6.6 | 6.0 | 510 | 18 | 4 |
| $Ca(NO_3)_2$ | 4.89 | 0.60 | 3.41 | 0.08 | 0.07 | 3.08 | 6.0 | 7.0 | 470 | | |
| *Experiment 4* | | | | | | | | | | | |
| $CaCl_2$ | 12.30 | 2.40 | 3.19 | | | 1.10 | | 6.8 | 710 | 18 | 4 |
| $CaCl_2$ | 61.70 | 5.70 | 7.24 | | | 1.30 | | 6.8 | 440 | | |

TABLE 3

*Absorption of ions from solutions of different magnesium salts**

| NAME OF SALT | INITIAL CONCENTRATION | IONS ABSORBED BY PLANT | | IONS ENTERING SOLUTION | | | REACTION | | $H_2O$ ABSORBED |
|---|---|---|---|---|---|---|---|---|---|
| | | Mg | Anion | Ca | K | $HCO_3$ | Initial | Final | |
| | *atm.* | *milli-equivalents* | | | | | *pH* | *pH* | *cc.* |
| $Mg(NO_3)_2$ | 21.1 | 4.75 | 5.40 | 0.70 | 0.03 | 2.48 | 5.0 | 6.7 | 130 |
| $MgSO_4$ | 20.7 | 1.82 | 1.17 | 0.60 | 0.06 | 0.08 | 5.0 | 5.3 | 135 |

* Age of plants was 28 days. Length of absorption period was 2 days.

## ABSORPTION OF POTASSIUM AND SODIUM FROM THE CHLORIDES AND SULFATES

The significant difference between the chloride and sulfate solutions in the amount of potassium removed seemed to be of sufficient interest to merit additional experimentation. In two experiments, the procedure was varied by adding a small quantity of calcium carbonate in suspension to the solutions of potassium salts (table 1, exp. 3 and 4). This prevented the increase of hydrogen-ion concentration consequent upon the more rapid absorption of potassium than sulfate. An appreciable concentration of calcium was also maintained in these solutions. The same result was obtained as before, that is, the potassium was absorbed from the chloride solution to a significantly greater extent than from the sulfate solution.

TABLE 4

*Absorption of ions from solutions of different nitrate salts**

(All values calculated in terms of milli-equivalents)

| NAME OF SALT | INITIAL CONCENTRATION | IONS ABSORBED BY PLANTS | | IONS ENTERING SOLUTION | | | | REACTION | | $H_2O$ ABSORBED |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $NO_3$ | Cation | K | Mg | Ca | $HCO_3$ | Initial | Final | |
| | *atm.* | *milli-equivalents* | | | | | | *pH* | *pH* | *cc.* |
| $Ca(NO_3)_2$ | 3.96 | 1.64 | 0.41 | 0.17 | 0.26 | | 1.60 | 6.8 | 7.2 | 370 |
| $KNO_3$ | 3.90 | 2.58 | 1.03 | | 0.28 | 0.24 | 1.76 | 6.0 | 7.2 | 430 |
| $Mg(NO_3)_2$ | 3.98 | 2.33 | 0.39 | 0.20 | | 0.37 | 2.18 | 5.4 | 7.2 | 435 |
| $NaNO_3$ | 4.14 | 2.06 | 1.55 | 0.39 | 0.24 | 0.28 | 1.48 | 6.6 | 7.0 | 410 |

* Age of plants was 19 days. Length of absorption period was 2 days.

Other experiments were made in which sodium salts were used, instead of potassium salts (tables 5 and 6). Here, also, the removal of cations from the sulfate solution was definitely smaller than from either the chloride or the bicarbonate solutions. In one experiment, sodium chloride and sulfate were mixed in equivalent quantities and the absorption of ions determined. The quantity of sodium removed was approximately the same as in the case of the chloride solution alone, but the removal of sulfate was apparently depressed by the presence of chlorine ions, since the percentage of sulfate removed was decreased and that of chlorine increased.

Referring to the solutions of sodium salts, sodium and chlorine were absorbed in practically equivalent quantities, but a greater number of equivalents of sodium than of sulfate were removed (a similar relation was observed between potassium salts). This discrepancy between the absorption of cations and anions is accounted for only partially by the change of reaction in the solution, but a somewhat greater displacement of calcium and potassium from the root tissues occurred in case of the sulfate.

TABLE 5

*Absorption of ions from solutions of different sodium salts**

| NAME OF SALT | INITIAL CONCENTRATION | IONS ABSORBED BY PLANT | | IONS ENTERING SOLUTION | | | | REACTION | | $H_2O$ ABSORBED |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Na | Anion | Ca | K | Mg | $HCO_3$ | Initial | Final | |
| | *atm.* | *milli-equivalents* | | | | | | *pH* | *pH* | *cc.* |
| NaCl | 10.70 | 2.78 | 2.75 | 0.30 | 0.13 | 0.25 | 0.75 | 6.2 | 6.4 | 670 |
| | 10.70 | 3.17 | 3.12 | 0.27 | 0.06 | 0.25 | 0.62 | 6.2 | 6.4 | 720 |
| $Na_2SO_4$ | 10.87 | 1.78 | 0.99 | 0.40 | 0.24 | 0.29 | | 5.0 | 4.2 | 660 |
| | 10.87 | 1.82 | 1.01 | 0.37 | 0.27 | 0.25 | | 5.0 | 4.0 | 730 |
| $NaHCO_3$ | 11.48 | 2.17 | 1.80 | 0.13 | 0.13 | 0.16 | | 7.9 | 7.8 | 680 |
| | 11.48 | 2.00 | 1.80 | 0.03 | 0.13 | 0.16 | | 7.9 | 7.8 | 690 |

* Age of plants was 17 days. Length of absorption period was 2 days.

TABLE 6

*Absorption of ions from solutions of different sodium salts*

| NAME OF SALT | CONCENTRATION | IONS ABSORBED BY PLANT | | | | AGE OF PLANT | LENGTH OF ABSORPTION PERIOD |
|---|---|---|---|---|---|---|---|
| | | Na | Cl | $SO_4$ | $HCO_3$ | | |
| | *atm.* | *milli-equivalents* | | | | *days* | *days* |
| NaCl | 10.57 | 1.91 | 2.36 | | | 31 | 4 |
| | 10.57 | 2.09 | 2.56 | | | | |
| $Na_2SO_4$ | 11.09 | 0.82 | | 0.66 | | | |
| | 11.09 | 0.82 | | 0.81 | | | |
| NaCl plus $Na_2SO_4$ | 11.31 | 1.91 | 1.83 | 0.25 | | | |
| | 11.31 | 1.96 | 2.03 | 0.23 | | | |
| $NaHCO_3$ | 11.09 | 1.35 | | | 1.00 | | |
| | 11.09 | 1.35 | | | 1.05 | | |

## ABSORPTION OF CHLORINE FROM DIFFERENT SALTS

One series of experiments was planned to determine the absorption of chlorine ions, when these were associated with different cations, calcium, sodium potassium and magnesium (table 7). No notable difference was observed, although the greatest quantity of chlorine was removed from the potassium chloride solution. The equivalents of potassium and of chlorine removed were similar, but the equivalents of chlorine absorbed were significantly greater than those of calcium or magnesium. Bicarbonate ions formed in the solution maintained the equilibrium between positive and negative ions.

TABLE 7

*Absorption of ions from solutions of different chloride salts**

| NAME OF SALT | INITIAL CONCENTRATION | IONS ABSORBED BY PLANT | | IONS ENTERING SOLUTION | | | | $H_2O$ ABSORBED |
|---|---|---|---|---|---|---|---|---|
| | | Cl | Cation | Ca | Mg | K | $HCO_3$ | |
| | *atm.* | *milli-equivalents* | | | | | | *cc.* |
| KCl | 6.32 | 1.97 | 2.05 | 0.60 | 0.20 | | 0.64 | 560 |
| $MgCl_2$ | 6.22 | 1.83 | 1.33 | 0.60 | | 0.16 | 1.16 | 550 |
| | 6.22 | 1.72 | 1.22 | 0.75 | | 0.16 | 1.12 | 595 |
| $CaCl_2$ | 6.37 | 1.69 | 0.80 | | 0.18 | 0.22 | 1.20 | 600 |
| | 6.37 | 1.58 | 0.60 | | 0.16 | 0.13 | 1.20 | 555 |
| NaCl | 6.32 | 1.64 | 1.97 | 0.60 | 0.16 | 0.31 | 0.72 | 575 |
| | 6.32 | 1.52 | 1.89 | 0.75 | 0.26 | 0.23 | 0.76 | 555 |

* Age of plants was 28 days. Length of absorption period was 2 days.

## ABSORPTION FROM PHOSPHATE SOLUTIONS

In a test of several phosphate solutions of similar concentrations of phosphate, equivalent absorption of anions and cations did not occur, calcium and magnesium being removed in smaller quantity than phosphate, and potassium in greater quantity (table 8). The amounts of phosphate removed from the different solutions were nearly the same, but the absorption was not sufficiently great in this series to warrant any exact comparisons. The decrease of hydrogen-ion concentration in the potassium phosphate solution was evidently caused by small amounts of bicarbonates derived from the roots.

TABLE 8

*Absorption of ions from solutions of different phosphate salts**

| NAME OF SALT | INITIAL CONCENTRATION | IONS ABSORBED BY PLANT | | IONS ENTERING SOLUTION | | | REACTION | | $H_2O$ ABSORBED |
|---|---|---|---|---|---|---|---|---|---|
| | | $PO_4$ | Cation | K | Mg | Ca | Initial | Final | |
| | *atm.* | *milli-equivalents* | | | | | *pH* | *pH* | *cc.* |
| $Ca(H_2PO_4)_2$ | 2.67 | 0.45 | 0.25 | 0.22 | 0.12 | | 3.7 | 5.6 | 395 |
| $KH_2PO_4$ | 2.73 | 0.43 | 0.62 | | 0.22 | 0.37 | 4.7 | 5.3 | 455 |
| $Mg(H_2PO_4)_2$ | 2.71 | 0.44 | 0.17 | 0.20 | | 0.47 | 4.0 | 5.4 | 385 |
| $NaH_2PO_4$ | 2.81 | 0.42 | | 0.41 | 0.24 | 0.32 | 4.7 | 5.4 | 475 |

* Age of plants was 19 days. Length of absorption period was 2 days.

## FURTHER STUDIES OF CALCIUM ABSORPTION

Additional studies were then made with calcium chloride, sulfate and nitrate. The results are given in table 2, experiments 3 and 4. The general results of the previous experiment were confirmed. In this instance, no determinable

absorption of sulfate occurred from the calcium sulfate solution, which was of lower concentration than before. Since calcium ions are apparently removed very slowly when in equilibrium with sulfate ions, it was decided to determine what would occur when nitrate ions were added (as potassium nitrate) to a solution of calcium sulfate. This addition of nitrate caused an increased absorption of calcium. While the total quantities involved were small, the difference noted was considerably beyond the limits of analytical error.

## GENERAL CONSIDERATIONS

All of the results of the experiments which have just been under consideration are consistent in showing that one ion can exert a definite influence on the absorption of another ion of opposite charge. It also appears that calcium, sulfate, and possibly magnesium ions are less readily removed from solution by barley plants than chlorine, potassium and nitrate ions. These same relations are found when plants are grown for long periods in culture solutions, such as those used by the writer. Under these conditions, calcium, magnesium and sulfate may be left in the solution in considerable quantity at the end of the period of growth, when all or nearly all of the nitrate, phosphate and potassium have been removed. In certain other experiments, not now reported, with Shive's solution, as well as that used by the writer, there was evidence that when solutions were changed at weekly intervals, no absorption of calcium, magnesium, or sulfate took place during certain periods, although potassium and nitrate were absorbed in appreciable amounts at all periods.

Some of the interrelations of cations in the processes of absorption have been considered in another paper (7), but one of the conclusions should be stated here. It was discovered that an effective concentration of sodium (0.1 *M* to 0.3 *M*) decreased the rate of absorption of calcium, magnesium and potassium. In the same way, potassium can influence the absorption of calcium. These facts illustrate the necessity for considering the possible inhibiting effect of one cation on another, at least within certain concentrations. A number of experiments bearing on this point are summarized in table 9. These data show quite definitely that sodium and potassium ions may be absorbed in appreciable quantities at the same time that calcium leaves the plants roots, so as to increase the concentration of this element in the solution. If the concentration of calcium is sufficiently high, however, absorption rather than excretion may take place. These experiments, supported by others reported elsewhere, clearly suggest that sodium and potassium ions may exert a depressing effect on the absorption of calcium ions.

The experiments so far under consideration were made with plants which were first grown in a complete culture solution and then transferred to single salt solutions. A number of other experiments were carried out in which the seedlings were placed in the solution to be studied immediately after germination. The data obtained by this procedure (table 10) show that the relative

rates of absorption of the different ions were very similar to those observed in the previous experiments.

The preceding statements bring up the question of the total capacity of a plant to remove ions from solution during a given period. To what extent is the absorption affected by the amounts of the ions already taken up by the

TABLE 9

*Effect of sodium and potassium salts on absorption of calcium*

| NAME OF SALT | INITIAL CONCENTRATION | | Ca ABSORBED OR EXCRETED | Na ABSORBED | AGE OF PLANTS | LENGTH OF ABSORPTION PERIOD |
|---|---|---|---|---|---|---|
| | Ca | Na or K | | | | |
| | *atm.* | *atm.* | *milli-equivalents* | | *days* | *days* |
| | | | *Experiment 1* | | | |
| $CaCl_2$ | 4.90 | | 1.00 | | 25 | 1 |
| $CaCl_2$–NaCl | 4.60 | 43.0 | −0.10 | 4.82 | | |
| $CaCl_2$–NaCl | 4.60 | 43.0 | −0.45 | 4.74 | | |
| | | | *Experiment 2* | | | |
| $CaCl_2$-NaCl | 4.59 | 43.1 | 0.60 | 5.50 | 21 | 3 |
| | | | *Experiment 3* | | | |
| $CaCl_2$-NaCl | | 34.8 | −0.55 | | 21 | 2 |
| $CaCl_2$-NaCl | 3.40 | 34.8 | 0 | | | |
| $CaCl_2$-NaCl | 6.30 | 34.8 | 0.70 | | | |
| $CaCl_2$-NaCl | 13.00 | 34.8 | 0.80 | | | |
| $CaCl_2$-NaCl | 25.40 | 34.8 | 1.60 | | | |
| | | | *Experiment 4* | | | |
| $CaCl_2$-NaCl | 0.85 | 0.87 | −0.45 | 0.43 | 25 | 2 |
| $CaCl_2$-NaCl | 4.20 | 4.35 | −0.15 | 0.96 | | |
| $CaCl_2$-NaCl | 9.20 | 9.50 | 0.50 | 1.22 | | |
| $CaCl_2$-NaCl | 16.80 | 17.40 | 0.80 | 1.65 | | |
| | | | *Experiment 5* | | | |
| $CaCl_2$-KCl | 0.04 | 0.66 | −0.75 | 0.47 | 26 | 2 |
| $CaCl_2$-KCl | 0.15 | 2.64 | −0.22 | 0.99 | | |
| $CaCl_2$-KCl | 0.31 | 5.30 | −0.31 | 1.45 | | |
| $CaCl_2$-KCl | 0.78 | 13.20 | −0.21 | 1.97 | | |

plant? The following experiment bears on this point (table 11). A large set of barley seedings was started in the usual manner in a complete culture solution and after a period of 10 days the solution was changed in the case of one half of the plants, while the other half was allowed to remain in the original solution. After 8 additional days of growth, all the plants received a change of solution and the absorption was then measured over a period of 2 days, according to

TABLE 10

*Increase or decrease of concentration of ions in various solutions as a result of absorption by barley plants**

| | COMPLETE CULTURE SOLUTION | | | | | | | | $CaCl_2$ SOLUTION | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | K | Ca | Mg | $NO_3$ | $PO_4$ | $SO_4$ | $HCO_3$ | Reaction | Ca | Cl | Reaction |
| | *milli-equivalents* | | | | | | | *pH* | *milli-equivalents* | | *pH* |
| Initial concentration | 2.26 | 9.48 | 4.85 | 10.38 | 1.13 | 4.98 | | 4.0 | 5.19 | 4.9 | 4.9 |
| Final concentration | 0.45 | 12.18 | 6.16 | 6.99 | 0.63 | 6.90 | 4.27 | 6.8 | 3.99 | 3.61 | 5.2 |
| Concentration at end in percentage of original concentration | 20.0 | 129.0 | 127.0 | 67.0 | 56.0 | 139.0 | | | 77.0 | 73.0 | |

| | $CaSO_4$ SOLUTION | | | $Ca(NO_3)_2$ SOLUTION | | | $KNO_3$ SOLUTION | | | $KNO_3 + Ca(NO_3)_2$ SOLUTION | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ca | $SO_4$ | Reaction | Ca | $NO_3$ | Reaction | K | $NO_3$ | Reaction | Ca | K | $NO_3$ | Reaction |
| | *milli-equivalents* | | *pH* | *milli-equivalents* | | *pH* | *milli-equivalents* | | *pH* | *milli-equivalents* | | | *pH* |
| Initial concentration | 7.08 | 7.07 | 5.4 | 6.23 | 5.58 | 5.2 | 4.51 | 4.89 | 5.0 | 2.60 | 4.47 | 7.08 | 5.2 |
| Final concentration | 5.99 | 5.83 | 4.0 (?) | 3.79 | 1.69 | 6.6 | 0.31 | 0.10 | 5.4 | 1.20 | 0.24 | 0.19 | 6.4 |
| Concentration at end in percentage of original concentration | 85.0 | 32.0 | | 61.0 | 30.0 | | 7.0 | 2.0 | | 46.0 | 5.0 | 3.0 | |

* Plants placed in solutions immediately after germination. Period of absorption 15 days. No additional water was added during this period.

TABLE 11

*Effect of preliminary treatment on absorption of ions during a given period**

| | K | Ca | Mg | $NO_3$ | $PO_4$ | $SO_4$ | REACTION | REMARKS |
|---|---|---|---|---|---|---|---|---|
| | *milli-equivalents* | | | | | | *pH* | |
| Initial concentration | 4.80 | 9.1 | 4.93 | 12.4 | 1.28 | 4.7 | 5.4 | |
| Final concentration | 1.77 | 14.1 | 7.97 | 12.1 | 1.52 | 8.0 | 6.8 | 1 preliminary portion of culture solution |
| | 0.69 | 13.6 | 7.80 | 9.0 | 1.43 | 7.5 | 6.8 | 2 preliminary portions of culture solution |

* No water was added during experimental period. Age of plants was 18 days and length of absorption period, 2 days.

the plan already described. The plants which had the initial change of solution were slightly larger, but smaller amounts of several ions were absorbed by these plants than by those receiving but one portion of solution. While these facts cannot be interpreted in terms of a definite chemical equilibria between the plant cells and the solution, it is plain that the rates at which ions can be absorbed may tend to decrease as a plant increases its content of these ions. The application of any simple chemical equilibria to these processes is obviously of doubtful practicality. The growth and multiplication of cells makes it impossible to subject systems of such complexity to any rigid analysis in this regard, at least in the present state of our knowledge.

## EFFECT OF CONCENTRATION ON ABSORPTION

The next consideration which is logically suggested concerns the effects of concentration on the absorption of ions. This is a phase of the inquiry which will require long extended investigation. One experiment can be presented now which may indicate one or two of the chief points involved. A series of solutions of $KNO_3$ plus $Ca(NO_3)_2$ of seven different concentrations was prepared with the potassium and calcium present in the same proportion in all solutions. Barley plants were transferred to these solutions after two weeks of growth. During the brief experimental period, no water was added to the cultures since it was desired to ascertain how the solutions would become adjusted when they were left undisturbed. The total absorption of water was noted. The results are shown in the form of three graphs (fig. 1). In each case, the solid lines indicate the actual concentration of the ion in question at the end of the period of absorption, and the dotted lines represent the increase of concentration which would have occurred if no ions had been removed by the plants. In the two lowest concentrations, the solutions have become more dilute with respect to nitrate, in the next higher concentration no change has taken place (that is, water and nitrate have been absorbed in equal proportion), while in the four solutions of highest original concentration, the residual solutions have increased in concentration, and the line for residual concentration approaches more closely to that for transpiration effect. A very similar condition was found in the case of potassium, except that only one solution became diluted. A much smaller proportion of calcium than of potassium or nitrate was absorbed and the two lines are close together. In one of the lower concentrations, calcium left the roots, instead of being absorbed.

This experiment, as well as others including several reported in an earlier paper (5), all lead to the conclusion that a much greater proportion of the total quantity of readily absorbable ions present in the solution is removed from solutions of low concentration. It follows from these statements that a plant possesses considerable ability to adjust itself to culture media of different concentrations.

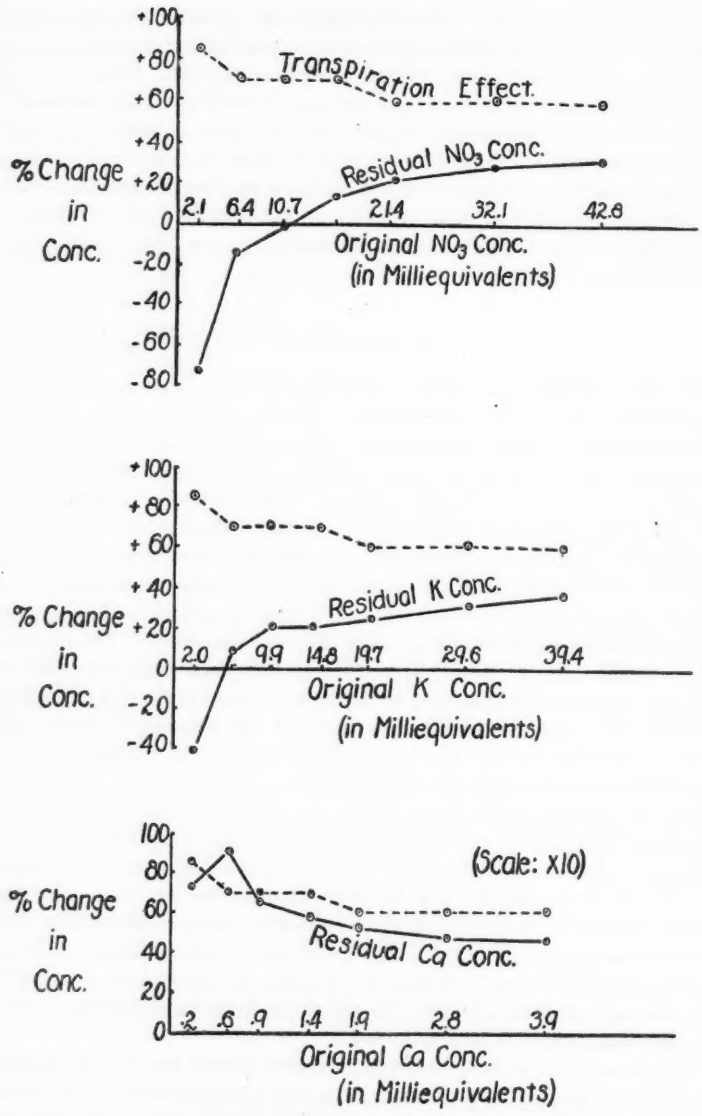


Fig. 1. Absorption of Ions from Solutions of $KNO_3$ and $Ca(NO_3)_2$ of Different Concentrations

Upper dotted line shows concentration which would have been produced by simple evaporation of water equal in amount to transpiration. Lower line indicates actual concentration of solution at end of absorption period (26 hours). Plants were 19 days old.

## RELATION OF ABSORPTION OF IONS TO ABSORPTION OF WATER

The relation of the intake of water to the intake of inorganic solutes has been a subject for speculation and experimentation from early times. It has been variously stated that the absorption of essential elements is entirely dependent on transpiration and that it is independent of the latter process. Careful quantitative experiments seem to be lacking in most cases. In a recent article by Muenscher (11), the literature of this subject is reviewed and additional data are presented. These refer chiefly to ash analyses, and so do not permit of any conclusions with reference to individual elements. It was shown, however, that in general, there was no correspondence between transpiration of water and ash content of plants.

In the present investigation, two methods of attack were adopted, first the reduction of transpiration by placing plants under bell jars (so arranged as to provide access of air) and second, the study of the effect of plants on the concentration of different ions in culture solutions, when no additional water was added during the course of the experiment. In other words, the relative absorption of ions and of water by the plants determined the change in the composition of the solution. One experiment of this type has just been described. In the majority of trials by the first method, the reduction in transpiration was not associated with anything like a proportionate decrease in the absorption of ions, although there was a general tendency for decreases in transpiration to be accompanied by decreases in the quantities of ions absorbed. In these experiments in which transpiration was reduced by placing the plants under bell jars, the conditions for growth or for metabolism (for example, the carbon dioxide supply) also may have been less favorable, which makes it very difficult to decide to what extent transpiration *per se* influenced absorption of ions. The data cited in table 13 show that in one experiment the solution from the plants kept in the open became less concentrated with respect to several ions than the solution from the plants placed under bell jars, even though in the former case the transpiration of water was nearly double.

It was thought that a more direct idea of the comparative intake of water and of inorganic elements might be obtained by allowing the solution to remain undisturbed during the brief period of the experiment. At the end of the periods, the solutions were analyzed without dilution. This simple procedure yielded some rather interesting results, which are reported in tables 10 and 12. The complete culture solutions became more concentrated with respect to certain ions, and more dilute with respect to others. The ions which regularly increased in concentration were Ca, Mg and $SO_4$. K and $NO_3$ ions were removed from solution more rapidly than water. The phosphate was increased in concentration under some circumstances, and under other circumstances decreased. The resultant effect on the culture solutions was that their composition was completely altered in a very short time. The nature of the alteration in the complete culture solution may be summarized as follows: Decrease in concentration

of K and $NO_3$ ions, increase of concentration of Ca, Mg and $SO_4$ ions, increase of $HCO_3$ ions, decrease in hydrogen-ion concentration. When growth and transpiration are restricted by placing seedlings in incomplete culture solutions, after a sufficient period of time, a decrease of concentration of Ca and $SO_4$ may also take place, as indicated in table 10. The different behavior of the three anions, $NO_3$, $SO_4$, and Cl, all in equilibrium with K ions, is illustrated in table 12.

Later an attempt was made to study the changes in the composition of a culture solution during a succession of very brief intervals. For this purpose, 80 culture vessels were used with a total of 560 plants. Samples of 5 cc. were taken from each of the culture vessels and united into compositive samples, which were analyzed. No additional water was added during the time of the experi-

TABLE 12

*Increase or decrease of concentration of various anions in equilibrium with K ions**

| | K | $NO_3$ | $SO_4$ | Cl | $HCO_3$ | REACTION |
|---|---|---|---|---|---|---|
| | *milli-equivalents* | | | | | *pH* |
| Initial concentration | 19.6 | 5.1 | 10.6 | 4.96 | | 5.2 |
| Final concentration | 24.9 | 0.9 | 15.9 | 5.64 | 4.64 | 6.9 |
| Concentration at end in percentage of original concentration | 127.0 | 18.0 | 149.0 | 114.0 | | |

* No water added during experimental period. Age of plants was 18 days and length of absorption period, 2 days.

TABLE 13

*Increase or decrease of concentration of ions under conditions of reduced transpiration**

| | K | Ca | Mg | $NO_3$ | $PO_4$ | $SO_4$ | $H_2O$ ABSORBED |
|---|---|---|---|---|---|---|---|
| | *milli-equivalents* | | | | | | *cc.* |
| Initial concentration | 4.55 | 8.0 | 4.4 | 11.4 | 1.26 | 7.2 | |
| Final concentration | 1.51 | 12.5 | 7.3 | 9.5 | 0.44 | 18.5 | 505 |
| Final concentration (under bell jar) | 3.15 | 9.6 | 5.2 | 9.7 | 1.43 | 13.1 | 265 |

* Age of plants was 19 days and length of absorption period, 2 days.

ment. The results as plotted are shown in figure 2. It is clear that the absorption of the different ions did not follow the same course. Again, it was found that the solution became more concentrated with respect to Ca, Mg and $SO_4$ ions, while the concentrations of K and $NO_3$ ions decreased. In this experiment, the $PO_4$ ion remained at approximately its original concentration. The sum of the equivalents of anions absorbed was greater than that of cations, bicarbonate ions being formed in the solution to maintain the equilibrium.

The effect of light was studied in one preliminary experiment (table 14). The usual technique was followed, except that during the absorption period, part of the plants were left in the light, and part placed under conditions of darkness. Of course, this involved a considerable difference in the amounts of water transpired. The concentration of all the ions, except K and $PO_4$ was

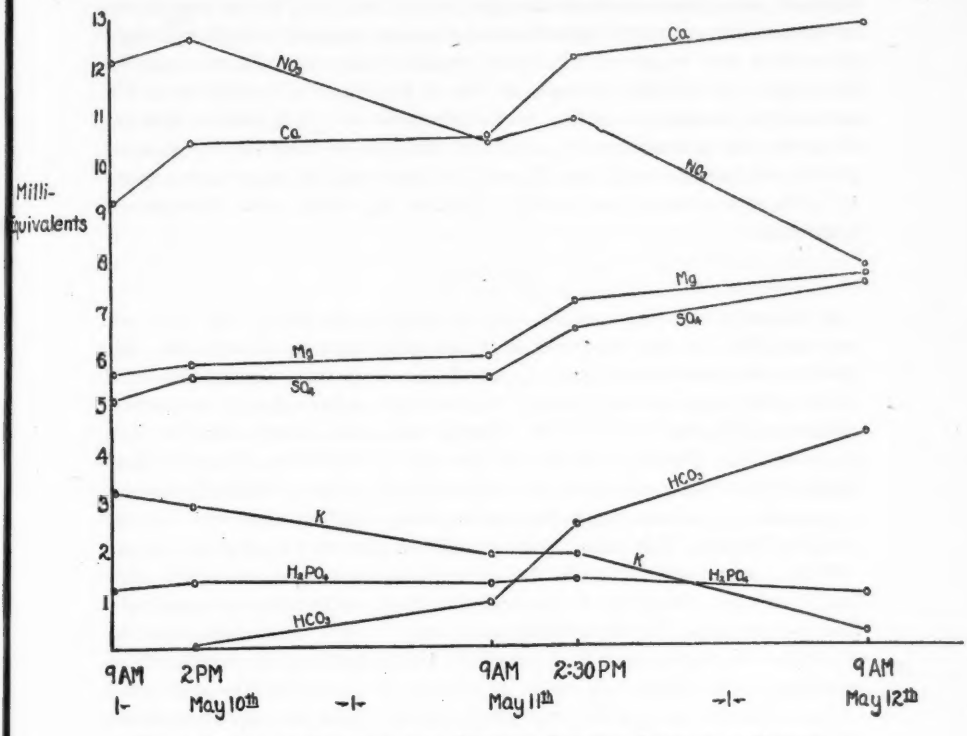


FIG. 2. CHANGE OF COMPOSITION OF COMPLETE CULTURES SOLUTION AT VARIOUS INTERVALS OVER A PERIOD OF 48 HOURS

Plants were 19 days old.

TABLE 14

*Effect of light on absorption of ions**

(All values calculated in terms of milli-equivalents)

| ION | CONCENTRATION IN LIGHT | | CONCENTRATION IN DARK | |
|---|---|---|---|---|
| | Initial | Final | Initial | Final |
| $NO_3$ | 10.5 | 10.9 | 10.5 | 8.1 |
| $SO_4$ | 4.7 | 8.8 | 4.7 | 5.4 |
| $PO_4$ | 1.3 | 0.04 | 1.3 | 0.70 |
| Ca | 8.7 | 11.8 | 8.7 | 9.1 |
| Mg | 5.2 | 9.3 | 5.2 | 5.9 |
| K | 4.4 | 1.2 | 4.4 | 1.7 |

* No water was added during the experimental period; 1090 cc. was absorbed in the light while 435 cc. was absorbed in the dark.

increased to a greater extent in the light than in the dark. In the case of the two ions mentioned, there was a decrease of concentration in the light, although more water was transpired. It seems probable that metabolic processes in which light was a factor, affected the rate of absorption of certain ions. Of course, from present information, no definite prediction of influence of light on absorption can be made for any particular case, since undoubtedly the stage of growth and previous nutritional history of a plant will be important factors. In other experiments, for example, nitrate ion might also decrease in concentration.

## DISCUSSION

In discussing in a more general way the results presented in this paper, we may conclude first that the processes of absorption have to do with ions. All the data we have obtained are in favor of this conclusion. Comber (3) in a recent article suggests that plants do not behave in soils as they do in solution cultures, in that they may absorb colloidal substances directly from the soil, or at least that diffusion may proceed through a continuous colloidal system formed by the intimate union of root hairs and soil particles. While this may be a possibility, it seems quite feasible to meet Comber's objections to soil solution theories. This point, however, will be discussed in another paper.

While it may appear probable that absorption processes are concerned with ions, it certainly should not be assumed that the ions are acting as completely independent units. On the contrary, as we have shown, one ion influences the absorption of another and in a complete culture solution, the complexity of conditions so far defies any really satisfactory analysis from the standpoint either of the chemist or of the plant physiologist. It does not appear, however, that anything is to be gained by interpreting the results of solution culture experiments in terms of salt proportion. In solutions of the dilution with which we are concerned, a high percentage of dissociation must exist. The actual analysis of such solutions must be made on the basis of ions or of chemical elements, and it is obvious that the results of absorption studies can best be expressed in these terms. Moreover, the interpretation of the data must almost necessarily be made in terms of ions or chemical elements.

Pantanelli (15) drew the general conclusion from his absorption studies that the two ions of a salt are seldom removed from solution in equal proportion, which conclusion is, on the whole, in accord with the data presented herein, although Pantanelli did not determine the extent to which an exchange of ions accounted for the discrepancies in the absorption of the different ions. Redfern (16) investigated the absorption by barley of calcium and chlorine ions from calcium chloride solutions. She found that calcium was absorbed in excess and other bases entered into solution. Our own results indicated that chlorine was absorbed by barley more rapidly than calcium. The difference between these two results can possibly be explained by the fact that the concentrations of solution used by Redfern were greater than our own. We have found in one

experiment with $MgCl_2$ that at a low concentration chlorine was absorbed more rapidly than magnesium, while at a relatively high concentration magnesium was removed to a greater extent than chlorine.

The replacement of one ion by another in a culture solution is a condition of any extensive inequality in the absorption of positively and negatively charged ions, since the change of hydrogen-ion concentration which can occur is comparatively limited. The notable example of replacement is, of course, that of $NO_3$ ions by $HCO_3$ ions. The ease with which this can occur makes it possible for $NO_3$ ions to be absorbed to a far greater extent than most cations. As already stated, the same type of replacement can occur with the chlorine ions, but to a much more limited extent. While the selective absorption of $NO_3$ has long been known, it does not appear that any one has advanced any really satisfactory explanation of the mechanism involved. In our earlier work, we held the view that positive and negative ions are either absorbed in equal proportion or else that an exchange of ions occurs. In the light of present knowledge, however, it is difficult to say whether, for example, K may be absorbed from $K_2SO_4$ as KOH, or whether the K ion may replace the H ion directly to the limited extent which corresponds to the increase of hydrogen-ion concentration taking place in the solution. When an alkaline solution is used and the hydroxyl-ion concentration of the solution decreased, it is possible that $HCO_3$ ion is involved in the selective absorption of bases.

It may be presumed that the rapid reduction which nitrate undergoes in the plant has something to do with its absorption, but this type of explanation would not answer for potassium which may also be absorbed with great rapidity. Experiments to be mentioned presently suggest that potassium occurs in plants largely in dissociated form.

The exchange of one base for another which seems to occur in root cells under certain conditions has been explained by True (18) as being attributable to reactions in the calcium pectate middle lamella. He emphasizes the importance of calcium in making other ions physiologically available. There can be no doubt that calcium is essential to the preservation of the normal permeability of plant cells, as has long been known from the work of Osterhout and others (14). It might be suggested, however, in the application of these facts to explain the absorption of ions by plants over extended periods of time, that an inadequate supply, not only of calcium, but of any essential element obviously will render the other elements present unavailable, since the plant will be unable to grow to any extent. It is often true, however, that lack of calcium may inhibit growth more quickly than lack of any other element. Different types of plants differ greatly in this respect. Barley seedlings grow well for some time and produce good root systems in solutions of $KNO_3$ (0.005 *M* concentration) without calcium, except that derived from the seed. Pea seedlings, on the other hand, grow well in solutions of calcium nitrate, but are soon injured by potassium nitrate. In the experiment with barley referred to in table 10, it does not appear that calcium in ordinary concentration had much effect on the absorption of potassium.

Another point of great interest is involved in the relative rates of penetration of different ions. Why, for example, is the $SO_4$ ion usually absorbed so much more slowly than other ions? It is not apparent that there is any simple relation between the ordinary chemical and physical properties of the ions and their rates of penetration into plant cells. Stiles (17) was unable to show any correlation between the rates of diffusion of $SO_4$ and Cl ions into gels and into living cells. It is quite apparent that not only the chemical characteristics of the ion, but also those of the cell membrane must be taken into consideration.

Certain other aspects of the problem of the absorption of ions by plants have been discussed elsewhere, but for the sake of completeness, a brief reference to the conclusions may be made at this point. Investigations (2, 8, 9, 13) on the fresh water alga *Nitella* from which cell sap may be obtained practically or entirely uncontaminated, have shown that most of the inorganic elements of the sap exist in dissociated form, and not chemically combined with organic substances. The cells contain ions in concentrations far higher than those of the media in which the plants grow. An expenditure of energy is apparently involved in the processes of penetration of ions into the cell sap. As possibly bearing on this relation, Davis and the writer have found (8) that Cl and other ions are not absorbed by *Nitella* to any extent except when the cells are exposed to light.

In the experiments reported in this paper, very brief periods of absorption were considered, but even in these brief periods, growth and metabolism were undoubtedly active, and the absorption of ions which occurred during the periods in question cannot be dissociated from these processes. The point of view which it is sought to suggest here is entirely different from that which assumes any simple type of chemical equilibrium between the plant and its culture medium. It cannot be doubted that the plant cell operates in accordance with the laws of chemistry and physics, but the system is a dynamic one, and the energy relations of the chemical reactions taking place are, as yet, unknown.

Possibly the initial event in the removal of an ion from solution may involve some sort of chemical combination betweeen the ion and a constituent of the cell wall or protoplasm. It was observed in connection with the experiments on *Nitella* (8) that nitrate ions penetrate more readily from an acidsolutionthan from an alkaline one. Evidence is also available from experiments on barley plants to show that from an alkaline solution cations are absorbed to a greater extent than anions, the hydroxyl-ion concentration being decreased (6). Perhaps some of these facts will suggest the applicability of Loeb's (10) recent researches on proteins, but in this connection it must be considered that it is possible for cations to be removed from solution more rapidly than anions, even when the reaction is acid, for example, in the case of potassium sulfate. It is also probable that compounds other than proteins play a part in the mechanism of absorption.

Finally, it must be very emphatically stated that the absorption of ions by a plant depends not only upon the culture solution, but upon conditions of light,

temperature, and humidity, and therefore it cannot be expected that any two experiments will give identical results, unless every condition is the same. Any experiments of the type described in this paper are useful only in so far as they throw some light on the general principles of ion absorption.

## SUMMARY

1. Determinations were made of the absorption of ions by barley plants from numerous solutions of single salts or simple mixtures of salts, as well as from complete culture solutions. Absorption was generally determined over very brief periods. Large numbers of plants were employed and comparisons were made between uniform sets of plants several weeks old.

2. Different ions were absorbed at different rates, but definite evidence was obtained of the significant influence of one ion on the absorption of another. All three types of relations were involved, cation to anion, cation to cation, and anion to anion.

3. The slowly absorbed sulfate ion decreased the rate of absorption of associated cations; thus potassium was absorbed much more rapidly from the nitrate or the chloride than from the sulfate. Similar relations were found between the ions of various other salts.

4. The presence of a relatively high concentration of sodium decreased the rate of absorption of other cations, particularly potassium and calcium.

5. During a given period the proportion absorbed from the total quantity of easily absorbable ions present decreases as the concentration of these ions in the solution decreases. In this way, plants can adapt themselves, to a considerable extent, to solutions of low concentration.

6. Ions are seldom removed from solution by the plant in the same proportion as water. Under some circumstances K, $NO_3$ and $PO_4$ ions may be removed from solution at a far greater rate than water. At the same time, Ca, Mg and $SO_4$ ions may be removed less rapidly than water. Therefore, as the plant absorbs water and ions, the same solution may become more dilute with respect to certain ions and more concentrated with respect to others.

7. The importance of climatic conditions as affecting absorption of ions is emphasized. One experiment is described in connection with the influence of light on absorption.

8. Attention is called to the fact that energy exchanges are involved in the processes of absorption. Permeability relations alone are inadequate to explain these phenomena in the living plant.

## REFERENCES

(1) Brooks, S. C. 1917 Methods of studying permeability of protoplasm to salts. *In* Bot. Gaz., v. 64, p. 230–249.

(2) Brooks, M. M. 1922 The penetration of kations into living cells. *In* Jour. Gen. Physiol., v. 4, p. 347.

(3) Comber, N. M. 1922 The availability of mineral plant food. A modification of the presence hypothesis. *In* Jour. Agr. Sci., v. 12, p. 363–369.

(4) DAVIS, A. R. 1921 The variability of plants grown in water cultures. *In* Soil Sci., v. 11, p. 1–32.

(5) HOAGLAND, D. R. 1919 Relation of the concentration and reaction of the nutrient medium to the growth and absorption of the plant. *In* Jour. Agr. Res., v. 18, p. 73–117.

(6) HOAGLAND, D. R. 1923 The effect of the plant on the reaction of the culture solution. Univ. of Calif. Agr. Exp. Sta. Tech. Paper (In press).

(7) HOAGLAND, D. R. 1923 The effects of salts on the intake of inorganic elements and on the buffer system of the plant. Univ. of Calif. Agr. Exp. Sta. Tech. Paper (In press).

(8) HOAGLAND, D. R., AND DAVIS A. R. 1923 The composition of the cell sap of the plant in relation to the absorption of ions. *In* Jour. Gen. Physiol., v. 5, p. 629–646.

(9) IRWIN, M. 1923 The behavior of chlorides in the cell sap of *Nitella*. *In* Jour. Gen. Physiol., v. 5, no. 4, p. 427–428.

(10) LOEB, J. 1922 Proteins and the Theory of Colloidal Behavior. McGraw Hill Book Co., New York.

(11) MUENSCHER, W. C. 1922 Effect of transpiration on absorption of salts by plants. *In* Amer. Jour. Bot., v. 9, p. 311.

(12) OSTERHOUT, W. J. V. 1917 Does the temperature coefficient of permeability indicate that it is chemical in nature? *In* Bot. Gaz., v. 63, p. 317–320.

(13) OSTERHOUT, W. J. V. 1922 Some aspects of selective absorption. *In* Jour. Gen. Physiol., v. 5, no. 2, p. 225–230.

(14) OSTERHOUT, W. J. V. 1922 Injury, Recovery, and Death, in Relation to Conductivity and Permeability. J. B. Lippincott Co., Phila. and London.

(15) PANTANELLI, E. 1915 Über Ionenaufuahme. *In* Jahresb. Wissen Bot. v. 56, p. 689–733.

(16) REDFERN, G. M. 1922 On the absorption of ions by the roots of living plants. *In* Ann. Bot., v. 36, p. 167–174.

(17) STILES, W. 1921 The penetration of electrolytes into gels. IV. The diffusion of sulphates in gels. *In* Biochem. Jour., v. 15, p. 629–635.

(18) TRUE, R. H. 1922 The significance of calcium for higher green plants. *In* Science, n. s., v. 55, p. 1–6.

(19) TRUE, R. H., AND BARTLETT, H. H. 1916 The exchange of ions between the roots of the *Lupinus Albus* and culture solutions containing three nutrient salts. *In* Amer. Jour. Bot., v. 3, p. 47–57.

(20) WAYNICK, D. D. 1918 The chemical composition of the plant as further proof of the close relation between antagonism and cell permeability. *In* Univ. of Calif. Publ. Agr. Sci., v. 3, p. 135–219.

# PARTIAL STERILIZATION OF SOIL, MICROBIOLOGICAL ACTIVITIES AND SOIL FERTILITY: II[1]

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The preliminary results presented in the first paper (16) have demonstrated that treatment of soil with volatile antiseptics and heat has an important influence not only upon the development of bacteria and protozoa, but also upon the soil actinomycetes and especially the soil fungi. The metabolism of soil fungi is of such a nature that by it we can explain readily the phenomena observed as a result of partial sterilization of the soil.

Among the chief disadvantages which present themselves in working with field soils is their great variability, both in regard to chemical composition and numbers of microörganisms, the large number of various environmental conditions influencing the numbers and activities of bacteria and the fact that the previous course of microörganisms in the particular soil, as far as numbers and activities are concerned, is unknown. Also, it was recognized that the determination of ammonia formation alone in the soil is not a reliable index of the activities of bacteria, first, because ammonia accumulation in the soil is subject to too many variables (15) and, second, because the fungi which develop rapidly in partially sterilized soil may either accumulate large quantities of ammonia or assimilate it and convert it into microbial protein, depending upon the amount of available energy in the soil and species of fungi developing as a result of partial sterilization.

An experiment was, therefore, carried out as follows:

One-kilogram portions of a uniform, air-dried, sieved Sassafras loam soil were placed in a series of sixty 1-quart glazed earthenware pots and the proper amount of moisture was added, amounting to 20 per cent of the weight of the soil (60 per cent of total moisture-holding capacity). The pots were then covered with glass plates and placed in the incubator at 25–28°C. Every 10 to 14 days, the soils were brought to weight by the addition of distilled water. At various intervals, 6 pots were sampled for the determination of nitrates and bacterial numbers. Samples were taken by means of a clean cork borer (about 1 cm. in diameter) to a depth of 3–4 inches from various parts of the pot. At the same time six of the pots were also placed in the respirator apparatus and the amount of carbon dioxide evolved, using a constant aeration for 14 days, was determined. The respiration was carried on at constant temperature (25–28°C.). At the end of the 14 days, the pots were taken out and covered again. The soils were otherwise undisturbed. These determinations of

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numbers of bacteria, actinomycetes and fungi, nitrate content and $CO_2$-producing capacity were continued until the changes became hardly noticeable within a brief period of time. For this a period of about 11 months was required. This procedure was followed in order to obtain a soil in which the history of microbial changes was known, a soil which had been brought to a definite level of activities (we may call it microbial balance or unstable equilibrium, as far as this term can be applied to microbial activities).

The different determinations made upon this undisturbed soil, when plotted, give us the courses of the changes of the microörganisms in the soil as indicated by the total numbers developing on the plate, nitrate accumulation and evolution of carbon dioxide. These curves are given in figure 1.

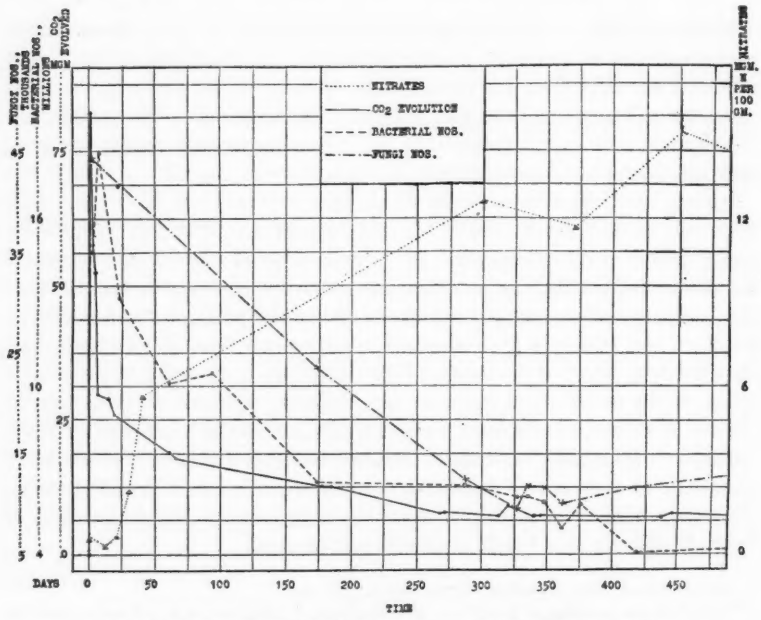


FIG 1. COURSE OF BIOLOGICAL ACTIVITIES IN UNDISTURBED SOIL

In view of the fact that the soil was air-dried before it was properly mixed and distributed in the pots, there is a decided increase in the numbers and activities of microörganisms, soon after moistening. In the case of bacteria, the maximum is obtained only after 3 or 4 days. This is followed by a drop in numbers, at first rapidly, then more slowly, so that after 175 days the numbers of bacteria and actinomycetes have come to a certain level at which the decrease in numbers is only very slow. The fungi reach their maximum soon after moistening and are then followed by a gradual decline, much less abrupt than in the case of bacteria and actinomycetes, and a certain level is reached only after about 250 days. The ratio of actinomycetes to the

total number of microörganisms developing on the plate declines with the increase in bacterial numbers, then increases with the decrease in the numbers of bacteria developing on the plate, till a constant ratio is attained, namely till about 20 to 25 per cent of the organisms, exclusive of fungi, developing on the plate are actinomycetes.

The rate of evolution of carbon dioxide reaches a maximum during the first 24 hours after moistening the soil, then it falls off very rapidly, reaching also a certain level after about 150 days. The more precipitous fall in the rate of evolution of carbon dioxide than the numbers of bacteria has been observed previously by Fischer (3) and others. Fischer explained this by the low respiratory power of the bacteria after they reached the maximum development, although they are still capable of developing into colonies on the plate. This phenomenon will be dwelt upon at greater length elsewhere. The nitrate content of the soil increases at first slowly, then rapidly, till a certain maximum nitrate content is attained, equivalent to about 10 mgm. of nitrate nitrogen in 100 gm. of soil. The curve in the case of nitrate accumulation in the soil is an autocatalytic curve, which has been previously pointed out by Miyake (10).

Eleven months after the soils were started, the numbers of microörganisms did not change appreciably. At this point a uniform balance of microbiological activities had become established, as indicated by the unchanging rate of $CO_2$ production. At this time the 60 pots of soil were divided into 16 groups, 3 or 6 pots in each group, and the soil in each pot was submitted to various treatments, as indicated below:

1. Remained untreated, to serve as control.
2. Soil was taken out from the pots, mixed thoroughly and placed in the pots again.
3. Soil was removed, well mixed with 0.5 per cent CaO, and returned to pots.
4. Soil was removed, well mixed with 1 per cent $CaCO_3$, and returned to pots.
5. Soil was spread out in large open glass dishes in incubator and allowed to air-dry for 14 days, then returned to original pots and brought back to original weight by addition of sterile distilled water.
6. Soils were treated as in group 5, but were reinoculated with 0.5 per cent of fresh soil (untreated soil from one of the control pots).
7. Soil from each pot was transferred to individual bottles, 1 per cent of toluene was added and bottles closely stoppered. After 48 hours, the soil was spread out for 3 hours to allow the toluene to evaporate; then it was returned to the original pot and sterile water added to bring back to weight.
8. Same as treatment 7, only reinoculated with 0.5 per cent of fresh soil.
9. Soil treated with 1 per cent of carbon bisulfide in a manner similar to the toluene treatment.
10. Same as treatment 9 but reinoculated with 0.5 per cent fresh soil.
11. Pots with soil placed in hot water, until soil temperature in center of pot reached 65°C., and kept at that temperature for 1 hour.
12. Same as treatment 11, but reinoculated with 0.5 per cent of fresh soil.
13. Soil removed from pots, 0.2 per cent of ground dry alfalfa hay was added to each and well mixed with the soil.
14. Soil removed from pots, 0.05 per cent of sulfur was added to each and well mixed in.

TABLE 1

*Influence of soil treatment upon the activities of protozoa**

| TREATMENT | AFTER 14 DAYS | | | AFTER 28 DAYS | | | AFTER 42 DAYS | | | AFTER 104 DAYS | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C† | F | A | C | F | A | C | F | A | C | F | A |
| Control | +++† | +++ | | ++ | +++ | +++ | +++ | ++ | +++ | | | |
| Mixed | +++ | +++ | | + | +++ | +++ | | | | | | |
| CaO | 0 | 0 | 0 | ++ | ++ | +++ | ++ | +++ | +++ | | | |
| $CaCO_3$ | + | +++ | | | +++ | +++ | +++ | ++ | | | | |
| Air dried‡ | 0 | ++ | 0 | | | | + | ++ | +++ | | | |
| Air dried and fresh soil | 0 | ++ | +++ | | | | ++ | +++ | ++ | | | |
| Toluene | 0 | 0 | 0 | ++ | +++ | +++ | ++ | ++ | ++ | | | |
| Toluene and fresh soil | ++ | +++ | | ++ | +++ | +++ | ++ | +++ | ++ | +++ | +++ | +++ |
| $CS_2$ | 0 | 0 | 0 | ++ | +++ | | | | | | | |
| $CS_2$ and fresh soil | +++ | | +++ | ++ | +++ | | | | | | | |
| Steamed | 0 | 0 | 0 | ++ | +++ | +++ | | | | | | |
| Steamed and fresh soil | ++ | +++ | ++ | +++ | +++ | | | | | | | |

* Protozoa were determined by adding 1 cc. of a 1 : 20 dilution of the soil to two flasks each containing 50 cc. of the sterile media (hay infusion and soil extract).

† C = ciliates, F = flagellates, A = amoebae; + = few, ++ = many, +++ = very many.

‡ Few flagellates after 14 days of air-drying.

15. Soil removed from pots, 200 mgm. of $K_2SO_4$ and 200 mgm. $CaHPO_4$ was added to each and well mixed in.

16. Same as treatment 15, with 250 mgm. of $NaNO_3$ in addition.

All the pots were then covered with glass plates and kept at optimum moisture in the incubator room. Since there was only enough room in the respirator for the study of $CO_2$ evolution from 12 soils at any one time and the period used was 14 days, the treatments were so arranged as to permit care of all the pots. At various intervals samples of soil were taken for the study of numbers of bacteria, fungi and protozoa, as well as nitrate content. Ammonia was determined only after prolonged incubation periods. The $CO_2$-production by the variously treated soils was also determined at various intervals. The results are presented in figures 2–16 while table 1 gives a record of the occurrence of protozoa.

## EFFECT OF TILLAGE

The mere mixing of soil, without allowing it to air-dry, resulted only in a slight stimulating effect as indicated by the carbon dioxide production (fig. 2). However, there was a depression in bacterial numbers. The protozoa were not affected. The fungi were at first depressed, then stimulated; the depression was accompanied by a reduction in nitrate content. Is it possible that the mere tilling of the soil, which means thorough aeration, resulted in the germination of the fungus spores with the development of a fungus mycelium? This might temporarily indicate a reduction in numbers followed by an increase when spore formation takes place. If that were the case, we could easily explain the assimilation of nitrate by the growing mycelium, increase in $CO_2$ production and reduction in bacterial numbers.

## INFLUENCE OF TREATMENT WITH CALCIUM OXIDE

The addition of 0.5 per cent CaO had a decided sterilizing effect upon the microbial population of the soil, as shown in figure 3. In view of the fact that the pots were placed in the respirator soon after the addition of the lime and kept in a $CO_2$-free atmosphere for 2 weeks, there was not enough of the $CO_2$ to carbonate the CaO. This accounts for the fact that no $CO_2$ is formed at first. It is also indicated by the very high alkalinity of the soil (pH = 9.6 +). The depression of $CO_2$ production was accompanied by a depression in the numbers of bacteria and fungi. However when the pots were exposed to the atmosphere and carbonation took place, there was a rapid increase in the numbers of microörganisms and $CO_2$ production. The protozoa were destroyed as a result of the treatment. But after 28 days large numbers of ciliates, flagellates and amoebae were found in the soil. This particular period coincided with the maximum development of bacteria and fungi as well as the maximum rate of $CO_2$ evolution. The rapid development of microörganisms brought about a decrease in the amount of nitrate in the

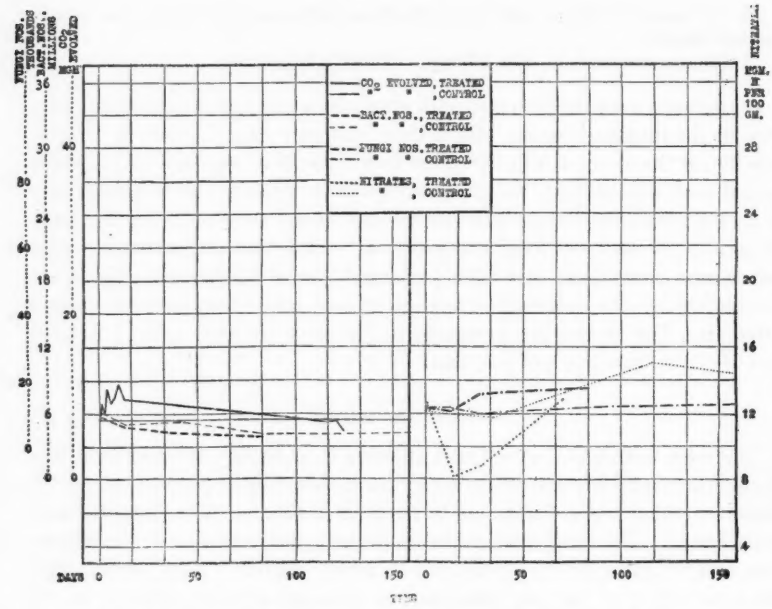


FIG. 2. INFLUENCE OF TILLAGE ON BIOLOGICAL ACTIVITIES IN SOIL

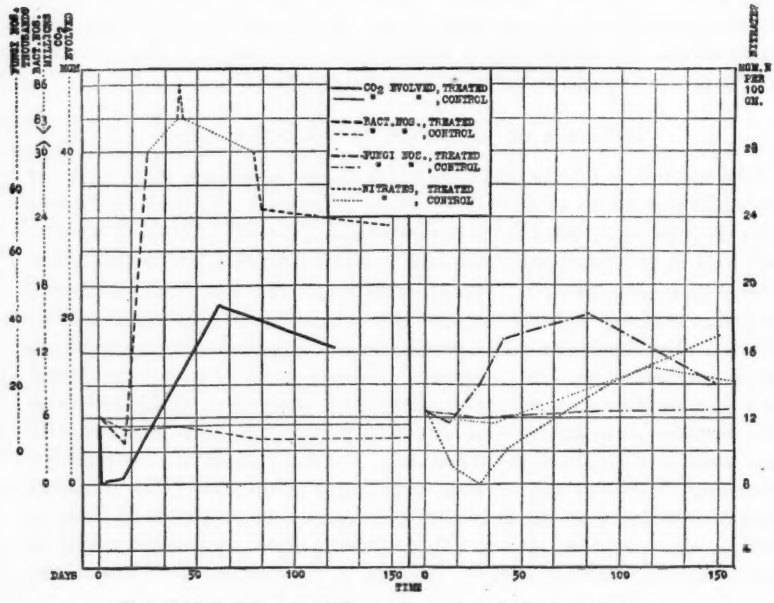


FIG. 3. INFLUENCE OF CaO ON BIOLOGICAL ACTIVITIES IN SOIL

soil, but as soon as the numbers of $CO_2$ production began to decline, an increase in the nitrate accumulation took place, so that in 150 days the amount of nitrate (16.9 mgm.) was higher than in the control. The soil contained only traces of ammonia, about 1 mgm. in 100 gm. of soil.

The influence of CaO upon the development of bacteria in the soil has been pointed out by various investigators. We need but refer to the work of Fischer (3), Hutchinson (5) and Miller (9). Fischer found that the addition of small quantities of CaO (0.1–0.3 per cent) brings about a temporary depression of bacterial numbers, soon followed by a decided increase. Larger concentrations of CaO have a more continued depressing effect, especially above 0.5 per cent, followed by a much greater increase in bacterial development, so that, 16 weeks after the application of 0.7 per cent CaO, the numbers of bacteria rose to 420 millions per gram. Equivalent concentrations of $CaCO_3$ gave only very slight increases in the number of bacteria, without any depressing effect. Fischer suggested that CaO does not act merely in neutralizing the acid condition of the soil, as in the case of the carbonate, but acts in a way stimulating bacterial life. These results were confirmed by Miller who showed that the greater the amount of CaO, the more prolonged is the checking effect upon bacterial development, but the greater will be the increase in bacterial numbers when it finally sets in. Five per cent of CaO completely prevents further growth of bacteria. The CaO has, however, a selective action upon soil bacteria, since *B. fluorescens* was not stimulated by 0.1 per cent of CaO and even destroyed by 0.3 per cent; 0.1 per cent of CaO diminished nitrification of ammonium sulfate, while 0.5 per cent stopped it almost completely.

The results of the above cited investigators are in conformity with our own results. The application of CaO to the soil has a pronounced effect in disturbing or even destroying the state of microbiological equilibrium. Hutchinson (5) found that its action is intermediary between the action of antiseptics and changes induced by high temperatures and it appears to bring about a decomposition of the organic nitrogen constituents of the soil.

The fact that protozoa develop rapidly in the soil even before the maximum development of bacterial activities set in, the fact that the development of fungi is very similar to that of bacteria, the fact that the $CO_2$-producing capacity of the soil is at a high level long after the protozoan development has attained a maximum, the fact that the nitrate content of the soil is depressed with the development of bacteria and fungi, then stimulated when those organisms begin to decrease,—all speak against any attempt to explain the phenomenon that has taken place by the destruction of protozoa. If there is any group of organisms that has suffered as a result of the treatment, it is the actinomycetes, which decreased from about 25 per cent of the population developing on the plate to 1–3 per cent and have not attained either their original numbers or percentage of total numbers even 150 days after treatment. This could hardly explain, however, the changes in biological activities, since in the case of some antiseptics, this depression is not so marked.

### INFLUENCE OF $CaCO_3$

The influence of $CaCO_3$ upon the microbiological activities in the soil is distinctly different from that of CaO, as shown in figure 4. There was a

marked evolution of $CO_2$ soon after the $CaCO_3$ had been added to the soil, not due to biological action but as a result of chemical reactions taking place in the soil between the $CaCO_3$ (1 per cent) and the acid reacting substances in the soil. The rate of $CO_2$ production soon drops to a low level, only slightly above the control. Bacterial numbers are stimulated only to a limited extent, similar to the results obtained by Fischer (3) and Miller (9). Fungi are at first depressed, then slightly stimulated. The addition of $CaCO_3$ to the soil does not result in a group of phenomena characteristic of partial sterilization, since there is no decided change either in the numbers or in the interrelationships of microörganisms (equilibrium) in the soil. A bacterial

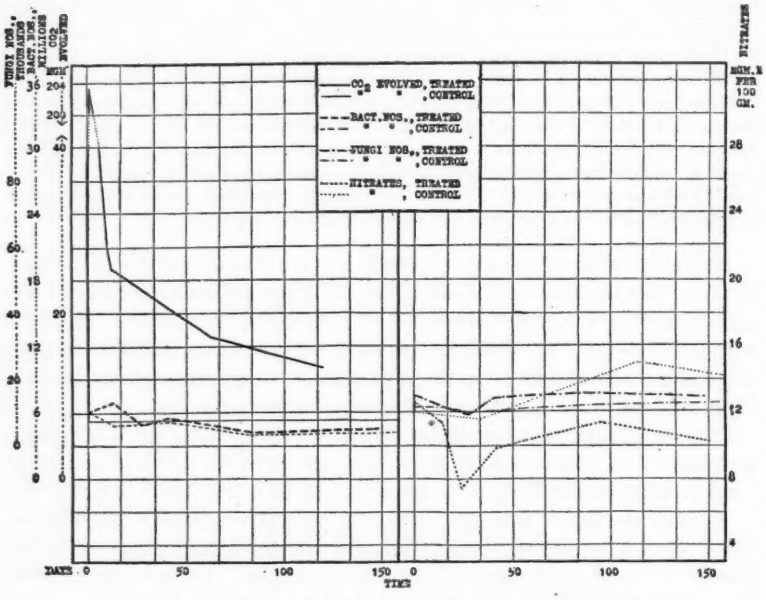


FIG. 4. INFLUENCE OF $CaCO_3$ ON BIOLOGICAL ACTIVITIES IN SOIL

plate from a soil treated with CaO shows a distinctly different picture from that treated with $CaCO_3$. In the former the minute pin-point, slowly growing colonies predominate with some large, watery, glistening white bacterial colonies; the plate is practically free from actinomycetes colonies. The $CaCO_3$ treated soil shows on the plate a normal bacterial flora, with a little more stress on the typical colonies of actinomycetes.

## INFLUENCE OF AIR-DRYING OF SOIL

Air-drying of the soil followed by moistening with distilled water to bring it back to optimum moisture, resulted in a decidedly stimulating effect upon

the activities of the microörganisms, as indicated by the increase in numbers of bacteria and fungi as well as $CO_2$ production (fig. 5). This was accompanied by a decided decrease in the amount of nitrates.

The increase in $CO_2$ production and diminution of nitrate content preceded somewhat the increase in numbers of microörganisms and the maximum of $CO_2$ production was attained before the maximum number of microörganisms. These two phenomena are probably correlated as follows: As soon as the air-dry soil is moistened, the bacteria begin to develop rapidly and the fungus spores germinate and develop into a mycelium; these activities, especially the synthesis of new cells, fungus and actinomyces mycelium involve a rapid

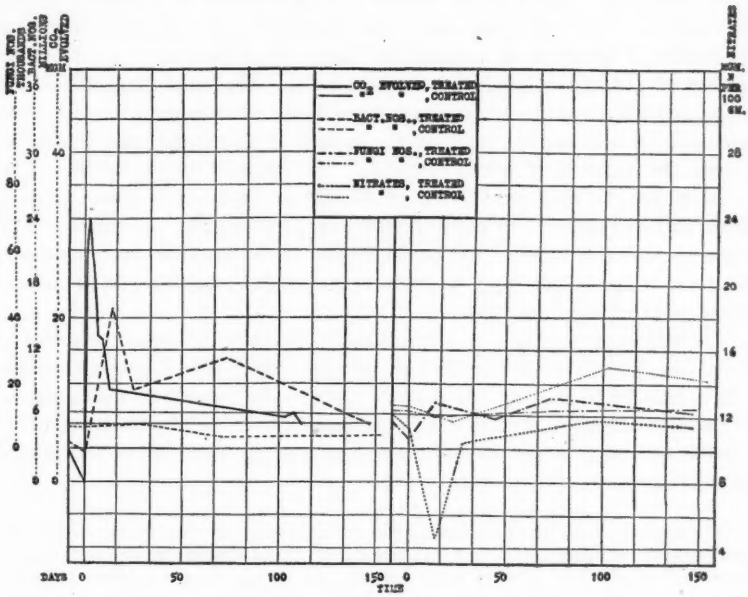


FIG. 5. INFLUENCE OF AIR-DRYING AND SUBSEQUENT REMOISTENING ON BIOLOGICAL ACTIVITIES IN SOIL

decomposition of organic matter and utilization of available nitrogen compounds (nitrates). The numbers of bacteria and fungi reach the maximum point only later, due to two influences: first, it is the development of bacteria and the maximum rate of reproduction which produce the $CO_2$ rather than the mere presence of the bacterial numbers as such; second, the greatest number of fungi may be indicated only after they have already completed their maximum growth, namely when they have gone into spores. Both of these phenomena will account for the fact that the maximum $CO_2$ production, which indicates the respiration or active growth of the organisms, precedes maximum numbers.

It is important to note that although air-drying of soil has a decidedly stimulating effect upon microbial activities in the soil, this effect is only of short duration: the numbers soon begin to fall off, the rate of $CO_2$ evolution soon reaches a maximum and the nitrate accumulation rapidly rises. Ammonia did not accumulate appreciably and, at the end of 100 days, there was only about 1 mgm. of ammonia-nitrogen in 100 gm. of soil.

The protozoa were not destroyed by the process of air-drying, the flagellates were as abundant as in the untreated soil, if not more so, while the ciliates and amoebae have disappeared temporarily; however, when the air-dry soil was inoculated with only 0.5 per cent fresh soil, the amoebae were also abundant soon after moistening. Surely the destruction of protozoa cannot account for the stimulating effect upon bacterial activities; one could just as readily, if not more so, explain the changes that have taken place by the course of growth of fungi and actinomycetes. The air-drying of the soil improved the physical condition of the soil and made organic matter more available as a source of energy to the growth of microörganisms; it also reduced the numbers of microörganisms. The bacteria and fungi were first to start growth resulting in $CO_2$ production and nitrate decrease; when a maximum of numbers was reached, they began to decrease. It remains to be seen whether this was due to the diminution of the available energy supply, to the development of fungi, to the increase in the proportion of actinomycetes, or to all of these combined.

The reinoculated pots gave almost exactly the same curves as the uninoculated (fig. 6); this speaks for the fact that the stimulation of bacterial activities is not due to the removal of any organisms, but merely to the change in physical condition of the soil and in the composition of the organic matter of the soil as well as to the shift of the temporary balance of the various groups of microörganisms in the soil.

The favorable influence of air-drying upon microbiological activities in the soil has also been observed by Rahn (12), Ritter (13), and others. Rahn obtained greater differences in heavy soils than in light soils, the differences were particularly marked in garden soils; this would tend to indicate that the stimulating effect of drying has to do with the modification of the organic matter in the soil; Rahn ascribed it to the degree of solubility of minerals. Ritter found that complete drying results in a greater stimulus than moderate drying; Ritter ascribed the favorable influence to a selective action upon the species of soil microorganisms. The fact that air-drying of soil brings about a decided increase in the solubility of the nutrients and change in colloidal condition of the soil has been pointed out by Kelley (7), Konig and associates (8). That air-drying of soil does not vitally affect the soil protozoa has been pointed out by Goodey and Greig-Smith.

The fact that air-drying modifies the organic matter of the soil making it a more available source of energy for microörganisms is clearly brought out in figure 7. The same soil was air-dried for 40, 358 and 519 days. At these intervals 1-kgm. portions were placed in pots, brought to optimum moisture and placed in the respirator. The longer the soil was air-dried the greater

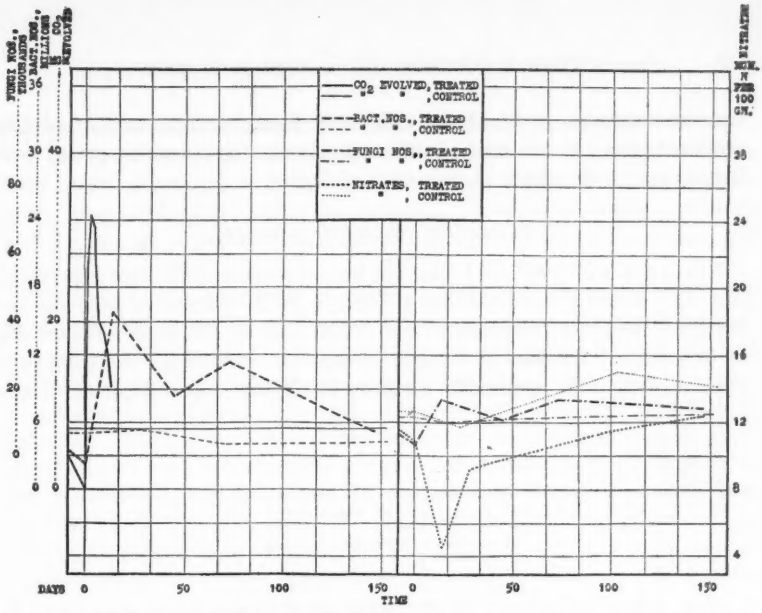


FIG. 6. INFLUENCE OF AIR-DRYING, SUBSEQUENT REMOISTENING, AND REINOCULATION ON BIOLOGICAL ACTIVITIES IN SOIL

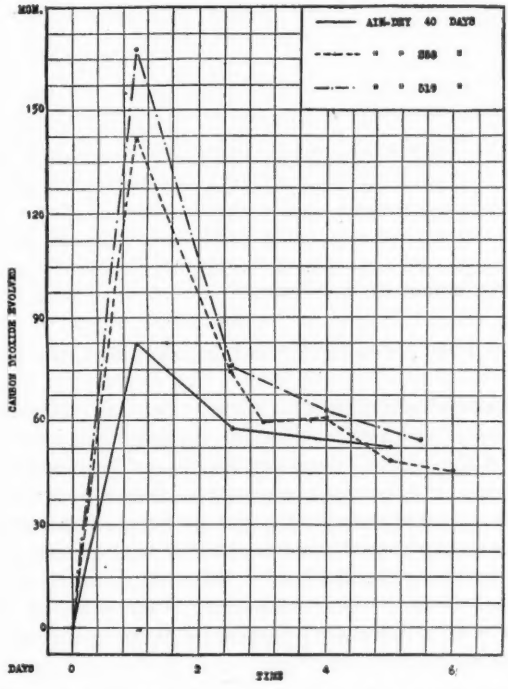


FIG. 7. INFLUENCE OF LENGTH OF TIME A SOIL IS AIR-DRY UPON ITS CARBON-DIOXIDE PRODUCTION UPON BEING REMOISTENED

was the maximum to which the $CO_2$ curve rose; the total amount of $CO_2$ produced from soils air-dried for different periods of time was also greater with the increase in the length of the period of drying.

### INFLUENCE OF HEATING OF SOIL

Heating of soil at 65° for 1 hour resulted in somewhat different phenomena (fig. 8). Heating also produced a decrease in the number of bacteria and fungi, followed by an increase; it resulted also in a rapid increase in the amount of $CO_2$ formed, followed by a rapid decrease; the protozoa disappeared and did not reappear in appreciable numbers for 28 days; the content of nitrates

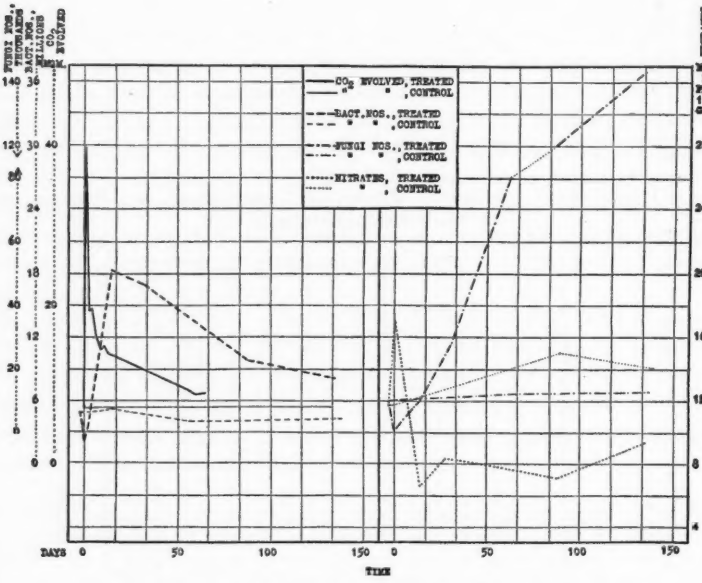


FIG. 8. INFLUENCE OF HEATING ON BIOLOGICAL ACTIVITIES IN SOIL

and other substances giving the characteristic reaction with phenol-disulfonic acid was increased as a result of heating, probably because of the complete transformation of the nitrites and possibly also of the ammonia present in the soil to nitrates; this increase in nitrate was followed by a drop accompanying the increase in the numbers of microörganisms. The bacterial numbers did not fall as rapidly as in the case of air-dried soils, but remained at a high level, even after a long period of time. However, the fungi did not increase rapidly, but only very slowly and continued to increase even after 80 days; the gradual course of increase of numbers of fungi in the soil is peculiarly parallel to the gradual fall of bacterial numbers; it is also important to note

that the nitrate content, after a slight increase, actually continued to decrease. The fact that we are not dealing here with protozoa is clearly indicated by their great abundance after 28 days treatment. Surely the large increase in numbers of fungi and soil transformations can be more readily correlated with the change in the bacterial numbers and nitrate content of soil than the presence of protozoa.

The ratio of actinomycetes to the total number of microörganisms developing on the plate was about 30 per cent before heating; this ratio dropped to about 15 per cent after heating, and about 3 per cent 2 weeks later; a gradual increase followed. In other words, the development of this group of organisms was greatly affected by heating of the soil: not only were the numbers reduced as a result of heating, but they did not increase until considerable time elapsed and even 4 months after treatment their ratio to the total number was only one-fourth of that before the treatment of the soil. The two phenomena, the rapid development of fungi and the slow, delayed growth of the actinomycetes as a result of soil treatment are fully correlated with the increase of bacterial numbers followed by a slow gradual decrease. It may be of interest to record that 57 days after the last determination reported in the curves the bacteria were on about the same level, the fungi began to decrease, while the nitrates nearly approached that of the control.

When the heated soil was reinoculated with 0.5 per cent of fresh soil (fig. 9), the protozoa began to develop much more rapidly, appearing abundantly after 14 days. The curve for $CO_2$ production was similar to the curve in uninoculated soil. The bacterial numbers reached a higher maximum, but began to drop much more rapidly; however, after 85 days they were on the same level with the uninoculated soil. The numbers of fungi behaved contrary to expectation, and contrary to the experiments reported in the previous paper; they remained consistently low for a long time and only $4\frac{1}{2}$ months after treatment did they show the marked increase which was observed in the other treated soils. This delayed development may be due to improper inoculation (only 0.5 per cent of soil used), to the destruction of the fungi capable of developing in the heated soil, or to the difference in the mycelial and spore development of the particular organisms. The nitrates decreased parallel with the increase of bacteria and began to increase with the drop in bacterial numbers. There was a much more rapid development of the nitrate content in the inoculated than in the uninoculated soil, due to the slow development of the fungi and the reintroduction of the nitrifying organisms. After 4 months, however, the nitrate accumulation is as rapid in the uninoculated as the inoculated soil. This may be due to the rapid development of the fungi which corresponds to a slowing down of the increase in nitrate accumulation as found in the uninoculated soil. The ratio of actinomycetes in the inoculated soil is similar to the ratio in the uninoculated. Two months after the last point on the curve, the fungi were found to be rapidly diminishing in numbers and the nitrate increasing. There was no appreciable increase

in the amount of ammonia in both heated soils, since the determination for ammonia was made several months after treatment (because of the scarcity of the soil) when the nitrifying bacteria became again active.

Steaming soils brings about a greater solubility of organic soil constituents, as pointed out by Richter [see Fischer (3)], Pickering (11), Elveden (2) and others. Fischer also observed a decided drop of the rate of $CO_2$ production following a rapid rise in inoculated steamed soils, while the bacteria remained at a high level. He explained this by the fact that, after a period of decided activities, the bacteria become more or less inactive, going

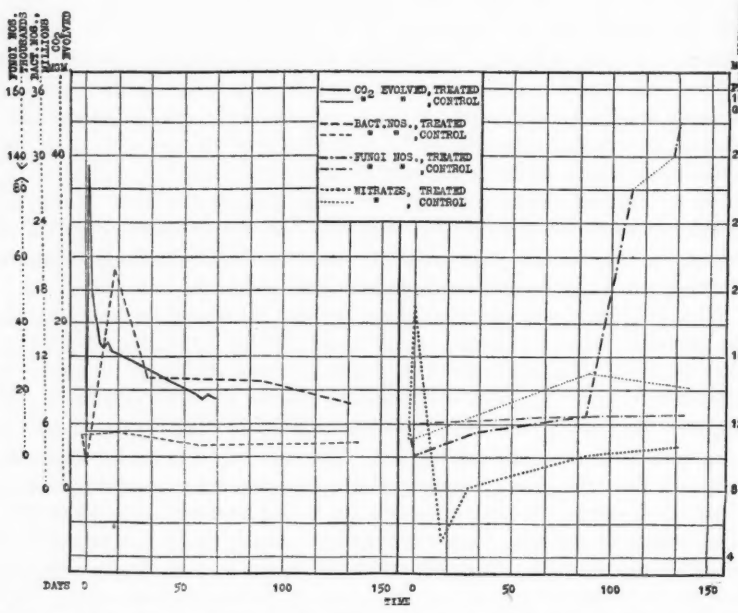


FIG. 9. INFLUENCE OF HEATING AND REINOCULATION ON BIOLOGICAL ACTIVITIES IN SOIL

into resting stages, which possess only a low respiratory power, but which are capable of developing into colonies on the plate. The stimulating effect upon bacterial activities was considered to be due to the decomposition of the bacterial bodies. Greig-Smith (4) suggested that the effect of heat upon soil is to destroy the bacterial toxins present there. Russell and Hutchinson explained it by the destruction of protozoa, although, as the figures show, the fungi curves have the same tendency as the bacteria curves, especially in reinoculated soils. Bolley (1) suggested that the destruction of disease-producing fungi and bacteria in the soil by heat has more to do with the increase in soil productiveness than any other phenomenon. Jachshevski (6) ascribed the cause of soil fatigue to fungi. The results in these experiments bear out the fact that fungi are destroyed by heat treatment; then those that survive (some typical soil fungi) reproduce rapidly to great abundance.

### INFLUENCE OF TOLUENE TREATMENT

Treatment of soil with toluene (fig. 10) brought about an increase in the rate of $CO_2$ production, followed by rapid decrease; this was accompanied by a decided increase in bacterial numbers; this increase remained at a high level for a long time before any appreciable decrease was observed and, even after 130 days, the numbers were still almost twice as high as in the untreated soil. The fungi were practically destroyed as a result of the treatment of the soil with the disinfectant and began to increase only slowly, then more rapidly; when they reached their highest point the fungi and the bacteria began to decrease, both remaining however at a much higher level than the control. Actinomycetes were only about 10 per cent of the flora developing on the plate instead of the 30 per cent in the control. The protozoa at first disappeared, then reappeared after 28 days. The nitrates were greatly diminished during and soon after treatment, but, after a prolonged period of time, they began to increase. No rapid multiplication of actinomycetes and fungi took place in this soil as was found in the case of a soil rich in organic matter (5A).

When the toluene-treated soil was inoculated with fresh soil, the rate of $CO_2$ production as well as the bacterial numbers rose above the uninoculated soil, but the bacterial numbers dropped down also more rapidly (fig. 11). The fungi did not reach such a high point as in the uninoculated soil, but after 130 days both soils had about the same number of fungi. The actinomyces ratio was about the same as in the uninoculated soil. The protozoa were abundant even after 14 days. The nitrates were also diminished rapidly during and soon after toluene treatment, but they began to increase sooner than in the uninoculated soil.

Several points need be emphasized here:

1. An increase in bacterial numbers is always sooner or later followed by a decrease. May it not be possible that, since in the reinoculated soil the maximum goes up higher than in the uninoculated, the drop is also sooner?

2. The increase in the number of fungi does not seem to depend primarily on the forms introduced but upon the capacity of the treated soil to support a fungus population. Among the fungi surviving toluene treatment we find most noticeabe the genera *Zygorhynchus*, soil Penicillium (pink-yellow form) *Cunninghamella* and *Cephalosporium*, all typical soil forms.

3. The nitrate bacteria are not completely destroyed by the treatment with toluene, they are only temporarily suppressed in the uninoculated soil, since they become always active again, sooner, of course, in the reinoculated soil.

4. Although all previous work noted (Russell and Hutchinson etc.), as well as our own results reported in the first part of this paper, indicate that toluene treatment of soil leads to an accumulation of ammonia, we did not find any increase in this particular case. This may be due to one or more causes (a) The ammonia was determined only after 120 days, since we did not want to remove from the pots any large quantities of soil which would be necessary for ammonia determinations, and at that time all the ammonia might have already been converted into nitrates. (b) In this particular instance the soil was rich in nitrates before treatment with the disinfectant; it is therefore possible that in this case the soil bacteria

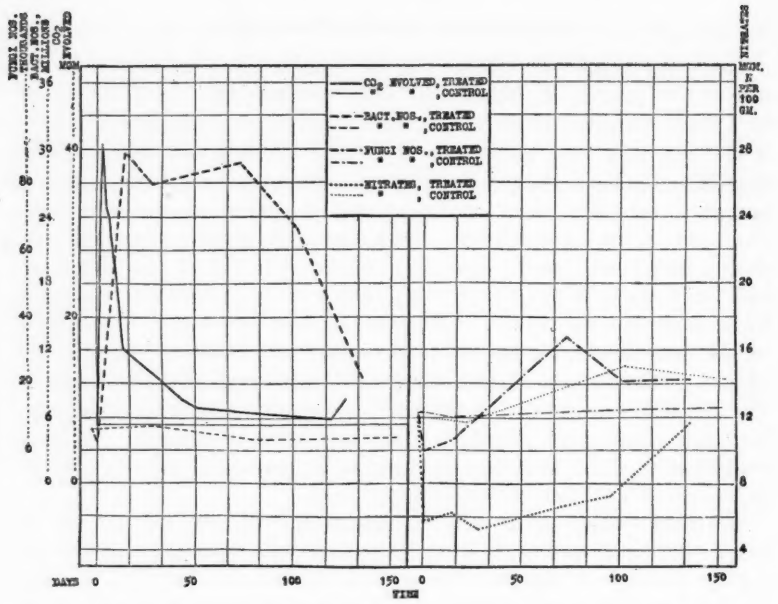


FIG. 10. INFLUENCE OF TOLUENE ON BIOLOGICAL ACTIVITIES IN SOIL

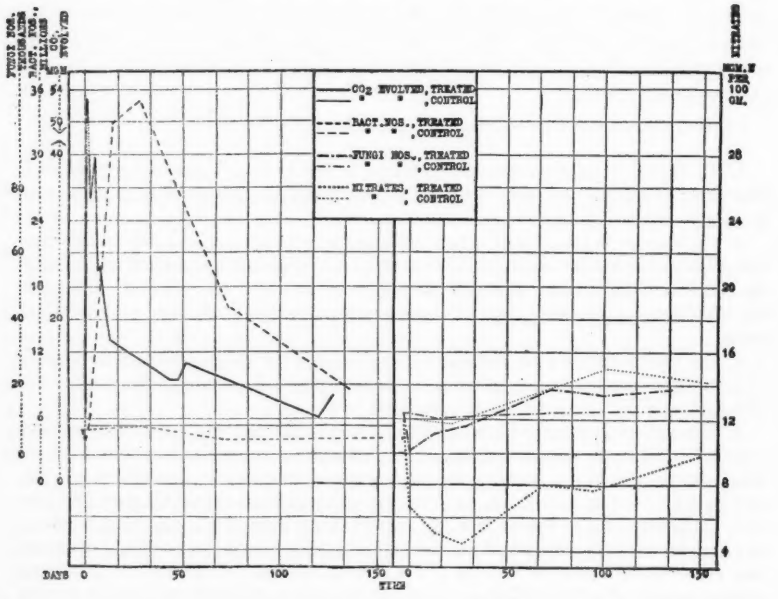


FIG. 11. INFLUENCE OF TOLUENE AND REINOCULATION ON BIOLOGICAL ACTIVITIES IN SOIL

and fungi did not break down readily the soil proteins and other nitrogenous compounds, but used the various carbon compounds (of a non-nitrogenous nature) as sources of energy using up the nitrate as a source of nitrogen and not liberating any ammonia. (c) It is also possible that in the period of 11 months that the soil was kept under favorable moisture and temperature conditions, all the readily decomposable nitrogen compounds were decomposed and the nitrogen finally converted into nitrate; the treatment with toluene therefore could not result in rendering a soil, which was not very rich in organic matter, containing no nematodes, worms and insects, and only comparatively small numbers of fungi, richer in available nitrogenous compounds.

### INFLUENCE OF CARBON BISULFIDE

Treatment of soil with 1 per cent of carbon bisulfide (fig. 12) resulted in phenomena very similar to the toluene treatment, heating of soil, air-drying, CaO treatment; the curves are, however, not only not identical but in some cases distinctly different, depending on the interrelationships of the various groups of organisms. The $CO_2$ production curve is quite similar to the other curves; protozoa were absent after 14 days and began to appear only after 28 days. Bacteria were at first depressed, then began to increase rapidly, reaching a maximum only after 90 days. The percentage content of actinomycetes was again reduced to a minimum and even after 120 days did not reach more than $\frac{1}{3}$ of the original proportion. The fungi were also depressed and soon recovered, but only very slowly; it is important to note that the rapid rise of numbers of fungi coincides almost exactly with the precipitous drop of bacterial numbers. The nitrate content in the soil was appreciably reduced as a result of treatment and soon after; in view of the fact that the nitrifying bacteria were put out of action, even if temporarily, there was no appreciable further increase in nitrate even after 120 days; the ammonia content, however, increased appreciably from less than 1 mgm. to over 3 mgm. per 100 gm. of soil.

In the case of the reinoculated soils (fig. 13), the same curve of $CO_2$ production is obtained with a more rapid rise in bacterial numbers. The rapid rise was also followed by a rapid fall. The protozoa were already evident after 14 days. These could hardly explain the precipitous fall of bacterial numbers, witness the almost coincident rise of fungous numbers. The fungi recovered in the reinoculated soil much more rapidly and began to rise precipitously after 35–40 days, this corresponding to the rapid drop in bacteria. The nitrate was here also reduced, but began to increase somewhat earlier than in the uninoculated soils, the gain was made partly at the expense of the ammonia in the soil. Determinations made 70 days after the last points on the curve indicated a drop in the numbers of fungi accompanied by a rise in the nitrate content of the soil.

The stimulating effect of disinfectants, especially carbon bisulfide upon the decomposition of organic matter in the soil has been pointed out previously by various investigators, using, as an index of the decomposition of organic matter, carbon dioxide production, oxygen absorption, and ammonia accumu-

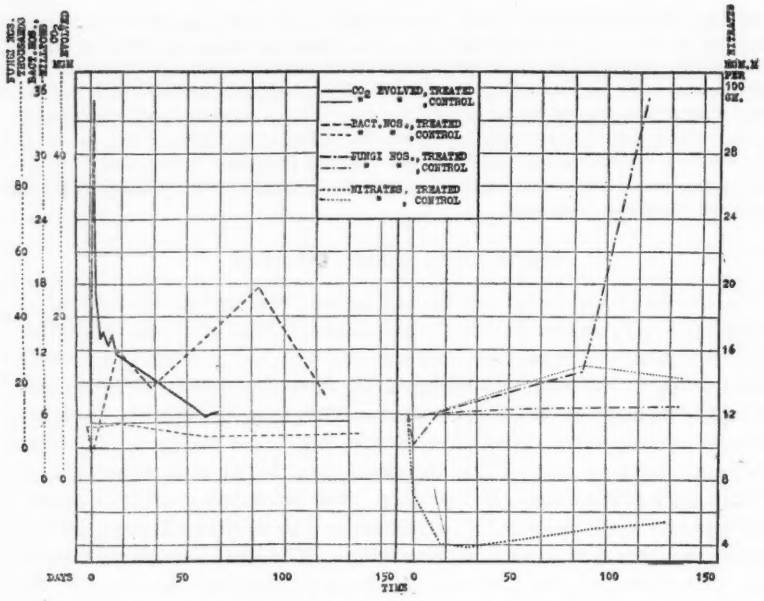


FIG. 12. INFLUENCE OF $CS_2$ ON BIOLOGICAL ACTIVITIES IN SOIL

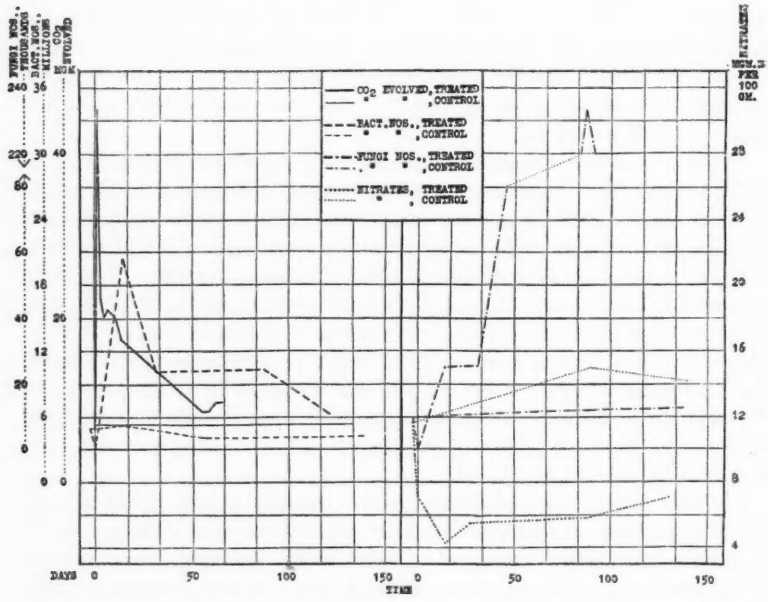


FIG. 13. INFLUENCE OF $CS_2$ AND REINOCULATION ON BIOLOGICAL ACTIVITIES IN SOIL

lation. While some ascribed this to the direct stimulating action of the disinfectant upon biological activities, others to the neutralization of soil toxins, modification of organic matter, change in physical condition of soil, Russell and Hutchinson ascribed it to the destruction of protozoa and Hiltner and Störmer to the destruction of the bacterial equilibrium in soil. According to the latter investigators, the disinfectant opens a way for an entirely new bacterial development, achieved by the unequal retardation and stimulation in the growth of the different groups of microörganisms; some become disproportionately prominent and others are almost entirely suppressed.

Stoklasa (14) suggested that the stimulating effect of the disinfectant is due to the destruction of a definite number of soil microörganisms; the surviving bacteria readily break down the dead organisms, liberating phosphate and other ions, which now become available for plant growth.

The following two curves, having nothing to do with disinfectants (although sulfur is looked upon as such by certain investigators) show the influence of available organic matter and of a small amount of sulfur upon the soil microbiological activities.

The addition of 0.2 per cent of ground dry alfalfa meal (2 gm. per 1 kgm. of soil) resulted in changes in the microbiological activities very similar to partial sterilization of soil (fig. 14). The curve for $CO_2$ production rose immediately, then fell precipitously. The rise in bacterial numbers followed soon after the rise in the $CO_2$ curve exactly as in the case of partial sterilization; the numbers remained at a rather high level for a long time, then decreased rapidly. The fungi rose rapidly, then began to diminish only slowly. The nitrates dropped rapidly, then began to increase. The curves in general are quite similar to the curves of steamed or toluene-treated soils that were reinoculated. The protozoa, of course, were not injured as a result of addition of readily available organic matter. This seems to suggest that the stimulating influence of heat and treatment with volatile antiseptics, outside of the destruction of fungi and other pests causing plant diseases, may be due to an increase in the available plant food in the soil either as a result of modification of the organic matter in the soil or destruction of various worms, protozoa, fungi, etc.

The addition of a small amount of sulfur (500 mgm.) to 1 kgm. of soil resulted (fig. 15) in a slight decrease in the numbers of bacteria, slight increase in the number of fungi and slight increase in the $CO_2$-producing capacity of the soil. The original decrease in nitrate content cannot be explained other than by the variability of the nitrate content in the particular soils. Fifty-four days after the last point on the curve was drawn, the sulfur-treated soils were again analyzed showing a steady decrease in the number of bacteria and increase in the number of fungi, the former dropping to 2,900,000 per gram and the latter going up to 25,000 per gram. The increase in soil acidity made the soil a poorer medium for the growth of bacteria and a better medium for the growth of fungi.

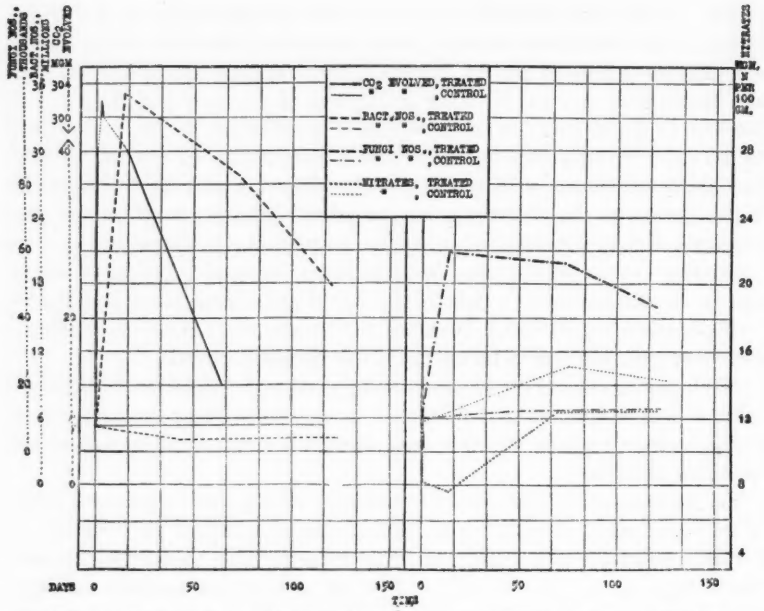


FIG. 14. INFLUENCE OF ORGANIC MATTER ON BIOLOGICAL ACTIVITIES IN SOIL

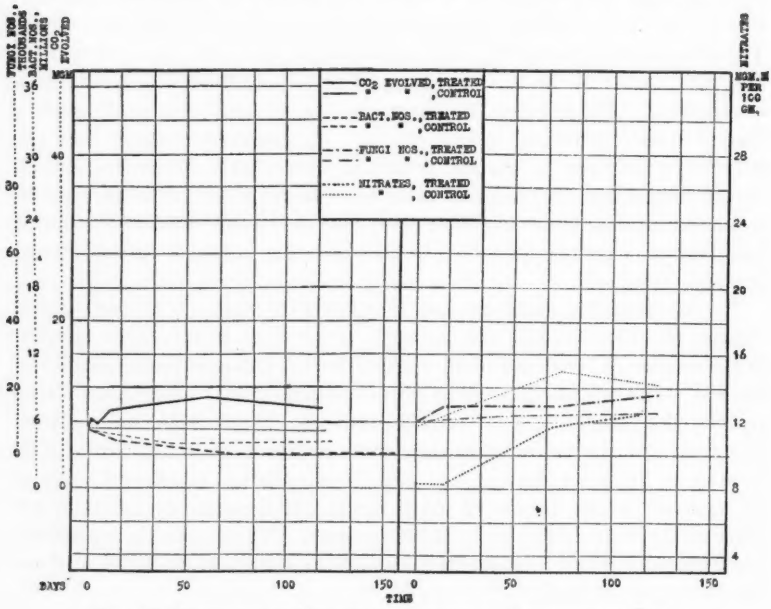


FIG. 15. INFLUENCE OF SULFUR ON BIOLOGICAL ACTIVITIES IN SOIL

The soils treated with $K_2SO_4$ and $CaHPO_4$ and these salts plus $NaNO_3$ did not show any different results from the ordinary cultivated (tilled) soils, so that they are not included here. This is probably due to the fact that the soils in question, due to their long incubation period, contained sufficient soluble minerals and nitrogen compounds, so that these did not become limiting factors.

Figure 16 indicates the total amounts of $CO_2$ given off by the soils the first week after treatment. The CaO treated soils are not included here since they gave off no $CO_2$ the first week. However, if the total $CO_2$ given off by the variously treated soils could have been determined, the CaO treated soils would have given as high results as any in the series, with the exception

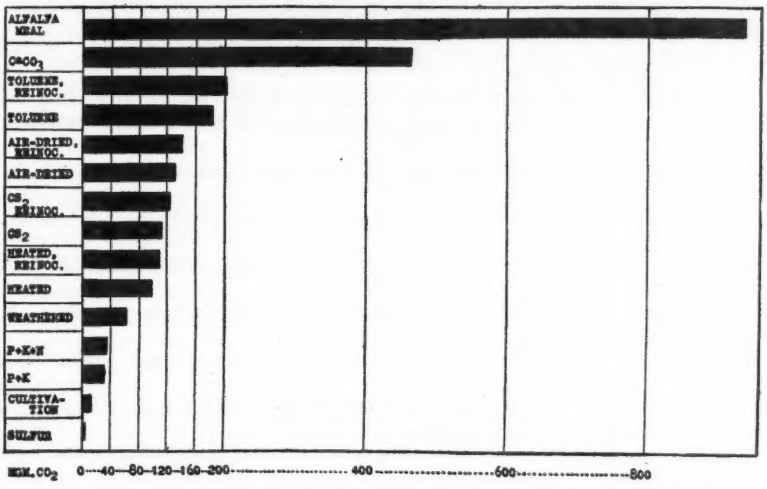


FIG. 16. CARBON-DIOXIDE PRODUCTION EFFECTED BY VARIOUS TREATMENTS—TOTAL $CO_2$ PRODUCED DURING FIRST WEEK AFTER TREATMENT

of the alfalfa treated soils. This is realized from the fact that the $CO_2$ remained at a high level in the CaO treated soils longer than in the case of any other treatment.

## SUMMARY

A soil with a known history as far as microörganisms and their activities are concerned has been prepared. The results of the influence of partial sterilization of soil by volatile antiseptics, heat, and CaO upon the numbers of bacteria, fungi, actinomycetes, protozoa and their activities in the soil thus prepared are reported. These are compared with the influence of air-drying, addition of $CaCO_3$, small amounts of organic matter and sulfur upon the same microbiological activities.

## REFERENCES

(1) BOLLEY, H. L. 1910–1913 Interpretation of results in experiments upon cereal cropping methods after soil sterilization. *In* Science, v. 33, p. 229–234; v. 32, 529–541; v. 38, p. 48–50, 249–259; N. D. Agr. Exp. Sta. Bul. 107.

(2) ELVEDEN, V. 1921 A contribution to the investigation into the results of partial sterilization of soil by heat. *In* Jour. Agr. Sci., v. 11, p. 197–210.

(3) FISCHER, H. 1909 Versuche über Bakterienwachstum in sterilisiertem Boden. *In* Centrbl. Bakt. (etc.), Abt 2, v. 22, 671–675.

(4) GREIG-SMITH, R. 1911 The bacteriotoxin and the agricere of soils. *In* Centbl. Bakt. (etc.), Abt 2, v. 30, p. 552.

(5) HUTCHINSON, H. B. 1913 The partial sterilization of the soil by means of caustic lime. *In* Jour. Agr. Sci., v. 5, p. 320–330.

(6) JACHSHEVSKI, A. 1912 On the causes which determine infertility of the soil and loss of crops. *In* Khosiaistvo (Kiev), v. 7, no. 34, p. 1103–1108; *cited in* Inter. Inst. Agr. Bul. Agr. Intel. Pl. Dis., v. 3, p. 2528–2531.

(7) KELLEY, W. P. 1915 Ammonification and nitrification in Hawaiian soils. Hawaii Agr. Exp. Sta. Bul. 37.

(8) KONIG, J., HASENBÄUMER, J., AND GLENK, K. 1913 Über die Anwendung der Dialyse und die Bestimmung der Oxydationskraft für die Beurteilung des Bodens. *In* Landw. Vers. Sta., v. 79–80, p. 491–534.

(9) MILLER, F. 1914 Über den Einflusz des Kalkes auf die Bodenbakterien. *In* Ztschr. Gärungsphysiol., v. 4, p. 194–206.

(10) MIYAKE, K. 1916 On the nature of ammonification and nitrification. *In* Soil Sci., v. 2, p. 481–492.

(11) PICKERING, S. U. 1910 The activities of heat and antiseptics on soils. *In* Jour. Agr. Sci., v. 3, p. 32–54, 258–276, 277.

(12) RAHN, O. 1908 Bakteriologische Untersuchung über das Trocknen des Bodens. *In* Centrbl. Bakt. (etc.), Abt. 2, v. 20, p. 38–61.

(13) RITTER, G. 1912–1913 Das Troknen der Erden. *In* Centbl. Bakt. (etc.), Abt. 2, v. 31, p. 116–143, v. 36, 346.

(14) STOKLASA, J. 1911 Biochemischer Kreislauf des Phosphat-Ions im Boden. *In* Centbl. Bakt. (etc.), Abt. 2, v. 29, p. 185.

(15) WAKSMAN, S. A. 1923 Microbiological analysis of soil as an index of soil fertility. IV. *In* Soil Sci., v. 15, p. 49–65.

(16) WAKSMAN, S. A., AND STARKEY, R. L. 1923 Partial sterilization of soil, microbiological activities and soil fertility. I. *In* Soil Sci., v. 15, p. 137–158.

# THE CHLOROSIS OF PINEAPPLE PLANTS GROWN ON MANGANIFEROUS SOILS

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Plants which do not make normal growth often develop a chlorotic condition of the leaves usually attributed to certain nutritional disturbances. Such a condition is more often noted on calcareous soils although other factors have been cited. The chlorosis of pineapple leaves on plants grown on the manganiferous soils of Hawaii is an example.

In an investigation of these manganiferous areas and the nutritional disturbances of numerous plants grown on these soil types Kelley (9) has reached the following conclusions. "From these evidences we may believe that the effects of manganese are largely indirect and are to be explained on the basis of its bringing about a modification in the osmotic absorption of lime and magnesia, and that the toxic effects are chiefly brought about through this modification rather than a direct effect of the manganese itself. . . . The percent of lime is increased while the absorption of magnesium and phosphoric acid is decreased. . . . in practically every instance a modification of the mineral balance was observed and this was found to follow the same direction. . . . regardless of whether the plant showed a toxic effect.

Gile (3) has noted a development of chlorosis on pineapple plants grown on calcareous soils in Porto Rico. He found the application of iron salts to the leaves to be very effective and to induce normal growth.

Johnson (8) on the basis of Gile's work applied solutions of iron salts to the leaves of pineapple plants suffering from chlorosis on the manganiferous areas in Hawaii and noted a return of the plant to normal growth. While he appears to have published no record of laboratory investigations he concluded that the chlorosis was due to a locking up of the iron by the manganese in an unavailable form in the soil. By a recalculation of a selected part of Kelley's data he then proceeded to substantiate his interpretation by showing a lower iron content in those plants which had become chlorotic. A study of Kelley's data *in toto* (9) does not indicate such to be true. The iron content of the ash of all plants analyzed shows considerable variation but no consistant relation to the manganese content of the soil. This is especially true of the pineapple plant of which Kelley made four analyses and of which only one, that cited by Johnson, showed an appreciably lower iron content in the plant grown on manganese soil as compared to that grown on a normal soil.

In a recent study on the nature of acidity in Hawaiian soils the writer has noted certain peculiarities which may throw some light on the chlorosis of pineapple leaves grown on these manganiferous areas. Hawaiian soils are usually acid in reaction although they are characteristically basic, that is very high in iron, aluminum and manganese oxides which are often in excess of silica. The iron content of these soils, expressed as $Fe_2O_3$, varies greatly and usually falls within the range of 20–30 per cent. This is true of the manganiferous areas as well as other types.

In a study of the relative acidity in manganiferous and non-manganiferous types, samples of soil and subsoil were taken representing both types. The acidity expressed as pH is given in table 1.

The manganese content of the nonmanganiferous samples was not determined but soils from the locality usually contain less than .5 per cent $Mn_3O_4$. The characteristic color of the manganese type is a chocolate brown changing to a red subsoil at 8–12 inches. The lower manganese content of the subsoil is typical. The subsoil samples represent the depth below change of color to two feet. With change of color there is also a notable change in physical texture. The top soil contains a higher silt and fine sand content and may be classed as a silty loam while the subsoil merges into a clay loam. This may be attributed to the deposition of manganese upon the soil grains thereby increasing their size. It is typical of the manganese type that the manganese is present as $MnO_2$ in the form of concretions or deposited as a film on the surface of the soil grains.

TABLE 1

*Showing acidity of two soil types expressed as pH*

| NATURE OF SOIL | SURFACE SOIL | | SUBSOIL | |
|---|---|---|---|---|
| | $Mn_3O_4$ content | Reaction | $Mn_3O_4$ content | Reaction |
| | *per cent* | *pH* | *per cent* | *pH* |
| Manganiferous | 7.3 | 5.9 | 3.5 | 6.5 |
| | 4.5 | 5.9 | 3.0 | 6.7 |
| | 4.7 | 6.0 | 3.9 | 6.7 |
| Nonmanganiferous | | 6.6 | | 6.5 |
| | | 5.9 | | 6.1 |
| | | 4.5 | | 4.3 |

The interaction between metallic elements in solution is governed, theoretically at least, by their relative positions in the electromotive series, each element replacing another standing lower in the series until a certain equilibrium is reached. The relative position of the more important elements present in the soil is as follows; potassium, sodium, calcium, magnesium, aluminum, manganese, iron. It must be admitted however that in a soil other factors such as hydrolytic action, basic replacement and double decomposition must be considered. Yet it is evident from the above that iron standing lowest in the series should be present in less concentration in the soil solution and that calcium and aluminum should exercise a greater displacing action than manganese, being higher in the series, and should further displace manganese itself.

In studying the relative solubility of iron and maganese in these acid soils it was found that iron salts were present in highest concentration in those soils of pH 5.5 or lower. That manganese is a factor principally in

those soils of pH 5.5 or higher. This applies to all types regardless of the actual manganese present in the soil. That is whether high or low.

Now with commercially grown crops here in the islands those which appear to produce normal growth on highly calcareous soils also grow with least disturbance on the manganiferous types. Reference is made to sugar cane and sisal (*Agave Sisalana*), more particularly the latter. Up until a recent date there were two sisal plantations on the island of Oahu. One was located in the coral areas practically devoid of soil and the other in the heart of the manganiferous areas. Plants grew normally in both but slightly better on the manganese soil due to better environment. Johnson has noted (8) that the calciphilous legumes are among the most strongly affected on manganese soils and claims that manganese is the cause of the chlorotic condition. He fails to point out however that *Crotolaria*, one of the most widely distributed legumes in the islands, grows luxuriantly on the manganese soils (9). This seems more tenable from Truog's work (10) in which he found all degrees of lime requirement in legumes as well as non-legumes.

Gile (3) found that it was possible to obtain a normal growth of pineapples on a soil containing approximately 30 per cent lime by heavy fertilization with stable manure. Kelley (9) found the same to be true on the manganese soils. The manganese present in Hawaiian soils is extremely soluble in organic acids. It hardly seems tenable that the fertility of these soils would be increased by heavy applications of organic fertilizers if the toxicity is due directly to manganese.

In studying the relation of iron, aluminum, manganese and lime to soil acidity in Hawaiian soils it was found that as compared to aluminum both iron and manganese appear to be only a small factor in determining the reaction. The aluminum content of these soils is approximately equal to that of iron but in measuring the solubility in dilute mineral acids and other weak solvents aluminum is dissolved in considerable excess. This also is in agreement with the respective position of these elements in the electromotive series. Also there is a direct relation between the solubility of lime and acidity. Table 2 shows the relation between the per cent of lime soluble in water saturated with $CO_2$ (shaking 1 part soil to 5 parts water) and the soil reaction.

Table 2 might be taken to indicate that lime is closely related to the concentration of the elements iron, aluminum and manganese in the soil solution of Hawaiian soils. Kelley (9) claimed that these manganese areas are of alluvial origin, that the manganese has been brought into solution by leaching and ultimately deposited in the low lying pockets. This theory is born out by the sporadic occurrence of the manganese spots which are usually lower than the surrounding non-manganiferous types. Judging from their respective positions in the electromotive series it is evident that the lime present in the soil solution is the principle factor in this deposition of manganese. It is usually present in excess of all other elements in the

soil solution and water extracts of Hawaiian soils. This further explains the lower hydrogen-ion concentration in the subsoils of manganiferous types. By replacement of manganese the concentration of calcium in the soil solution is increased. Because of the loose texture of this soil type this solution passes easily from the top soil into the subsoil. The humus content of these soils is very low. The hydrogen-ion concentration appears to be due principally to the presence of acid-reacting silicates. The lime is fixed in the subsoil by these acid aluminum silicates forming silicates of lesser acid tendencies. On determining the solubility of lime in $CO_2$-saturated water the results shown in table 3 were obtained with the three manganese soils and subsoils.

These results appear to indicate the presence of silicates of lesser acid tendencies in the subsoil. It will be noted that the solubility in the subsoil is lower than the soil yet the hydrogen ion concentration is lower. The

TABLE 2

*Relation of lime to pH values of typical Hawaiian soils*

| Soil No. | 848 | 367 | 399 | 765 | 186 | 621 | 722 | 872 | 3 |
|---|---|---|---|---|---|---|---|---|---|
| Per cent of CaO | 0.008 | 0.009 | 0.013 | 0.015 | 0.017 | 0.023 | 0.033 | 0.081 | 0.131 |
| pH values | 4.63 | 4.88 | 4.97 | 5.66 | 5.73 | 5.98 | 7.00 | 7.67 | 8.01 |

TABLE 3

*Relation of solubility of CaO in soil and subsoil*

| | SOIL 1 | | SOIL 2 | | SOIL 3 | |
|---|---|---|---|---|---|---|
| | Top soil | Subsoil | Top soil | Subsoil | Top soil | Subsoil |
| Per cent of CaO | 0.023 | 0.017 | 0.015 | 0.010 | 0.028 | 0.022 |
| pH values | 5.9 | 6.5 | 5.6 | 6.7 | 6.0 | 6.7 |

higher solubility of lime in the manganese soils as compared to the normal red soil is typical. This greater solubility of lime, the activity toward the replacement by calcium of manganese, aluminum and iron in solution, the open texture and good aeration in such types which assures a ready supply of air and carbon dioxide for the solution of calcium as bicarbonate appear to be important factors in the chlorosis of pineapple plants grown on this soil type. It might also be mentioned at this point that pineapple roots are almost entirely confined to the surface soil. Also that the development of chlorosis usually follows liming even on the more acid island soils.

Wilcox and Kelley (11) in an anatomical examination of the physiological disturbances in plants grown on manganese soils noted a superabundance of calcium oxalate crystals in the chlorotic pineapple leaves. The chemical analysis of leaves has also shown a markedly higher lime content as compared to the normal green leaves.

## AVAILABILITY OF IRON

Comber (2) has developed a qualitative test for soil acidity in which he shakes the soil with a solution of KSCN in alcohol. By increasing the concentration of $Fe(SCN)_3$ in the liquid phase by the use of this solvent he obtains a very delicate test for iron indicating the presence of acid reacting salts of this element and aluminum. The delicacy of this test is further increased by using an ether-alcohol solvent for the reagent. As thus applied to the manganese soils all showed a positive test for iron in solution. The depth of color is just as great as in the nonmanganiferous soils of equal hydrogen-ion concentration. One difference was noted however. The color of the test gradually fades in those soils of pH 6.0 or higher to a greenish blue. This is due to the presence of manganese as dioxide and not to the presence of manganese salts in solution. This was noted in the normal soils containing as low as .4 per cent $MnO_2$ and was produced in a soil of pH 4.3 by the addition of $MnO_2$ but not by the addition of any amount of manganese salts.

Comber (2) attributes this reaction to a double decomposition while Carr (1) suggests the actual solution of iron and aluminum which existed previously as colloidal basic salts. In either case the test indicates the presence of iron in readily available form in the manganiferous type and that $MnO_2$ does not hold the iron in an unavailable combination.

## MOBILITY OF IRON

In a very thorough study of the availability of iron and its mobility in the plant, Gile and Carrero (4, 5, 6, 7) have shown certain factors to greatly influence the mobility of iron in the leaves. They found that chlorotic leaves on calcareous soils when restored to normal color by spraying with iron salts do not transfer this iron to new leaves but the new leaves must be sprayed in order to maintain the green color of the plant as a whole. That is to say, with the withering of the old leaves the iron is not transported to the new. In the commercial spraying of pineapple plants of Hawaii which is successfully practised on an extensive economic scale, plants grown on all soils show a response to spraying with solutions of ferrous sulfate. Of course, this is most marked on the manganese soils where the chlorotic condition is more prevalent. Also the spraying must be continued at intervals throughout the life of the plant. These facts clearly indicate the low mobility of iron even in the normal pineapple plants.

## CONCLUSIONS

It is believed that the forgoing results indicate that the chlorosis of pineapple leaves on plants grown on manganese soils is due to a greater assimilation of lime indirectly caused by the presence of manganese in excessive amounts in the soil.

That the principal physiological disturbance is the greater immobility of iron in the plant resulting from the excessive lime content of the leaves and stalk and the low rate of mobility of iron even in normal pineapple leaves.

Iron is shown to be present in equally available form in both manganiferous and nonmanganiferous soil types of equal hydrogen-ion concentration. The fact that the addition of soluble iron salts to the soil is without effect and that new leaves must be sprayed indicates lack of mobility in the plant to be the principle inhibiting factor.

It is not denied that manganese under certain conditions may exert a toxic effect on plants. Numerous references in literature tell of such. This is especially true in water and sand cultures carried on in the absence of the complexities of soil environment. Also it is recognized that manganese may displace iron in solution in water and sand cultures. The relative positions in the electromotive series proves this, but it also indicates that lime functions as such with relatively greater activity.

## REFERENCES

(1) CARR, R. H. 1921 Measuring soil toxicity, acidity and basicity. *In* Jour. Indus. and Engin. Chem. v. 13, p. 931–933.

(2) COMBER, NORMAN M. 1920 A qualitative test for sour soils. *In* Jour. Agr. Sci., v. 10, p. 420–422.

(3) GILE, P. L. 1911 Relation of calcareous soils to pineapple chlorosis. Porto Rico Agr. Exp. Sta. Bul. 11.

(4) GILE, P. L., and CARRERO, J. O. 1914 Assimilation of colloidal iron by rice. *In* Jour. Agr. Res., v. 3, p. 205–210.

(5) GILE, P. L., and CARRERO, J. O. 1916 Immobility of iron in the plant. *In* Jour. Agr. Res., v. 7, p. 83–87.

(6) GILE, P. L., and CARRERO, J. O. 1916 Assimilation of iron by rice from certain nutrient solutions. *In* Jour. Agr. Res., v. 7, p. 503–528.

(7) GILE, P. L., and CARRERO, J. O. 1917 Absorption of nutrients as affected by the number of roots supplies with the nutrient. *In* Jour. Agr. Res. v. 9, p. 73–95.

(8) JOHNSON, MAXWELL, O. 1917 Manganese as a cause of the depression of the assimilation of iron by pineapple plants. *In* Jour. Indus. and Engin. Chem., v. 9, p. 47–49.

(9) KELLEY, W. P. 1912 The function and distribution of manganese in plants and soils. Hawaii Agr. Exp. Sta. Bul. 26.

(10) TRUOG, EMIL 1918 Soil acidity: I. Its relation to the growth of plants. *In* Soil Sci. v. 5, p. 169–195.

(11) WILCOX, E. V. and KELLEY, W. P. 1912 The effect of manganese on pineapple plants and the ripening of the pineapple fruit. Hawaii Agr. Exp. Sta. Bul. 28.

# THE EFFECT OF MOISTURE ON SOIL COLOR

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The color of soils is one of the most important characteristics upon which their classification depends. But soil colors are not readily determined. There are no definite standards which may be utilized as a basis for comparison when soils are mapped.

In an earlier publication (1) attention was called to the difficulties which soil surveyors encounter in the field, when they are mapping, because of color variations in soils and uncertainty regarding the exact meaning of the terms used in color descriptions. It was shown in a study of several soil types, that descriptions of soil colors, as given by different men, vary considerably, particularly when light colored soils are described. The personal equation seems to play an important part in the descriptions. But there are other factors involved. The lack of a definite terminology for soil colors causes a groping for the right descriptive terms and hence long uncertain descriptions are often given especially for light colored types. Then too, the seasonal conditions seems to play a part for the same man may describe the same type somewhat differently in a different season. Hence the question is raised whether descriptions of soil colors should be based on air-dry samples or on field observations.

The official descriptions of soil series as given by the Bureau of Soil are based on air-dry samples but the soil survey reports give the color descriptions devised by the field men from their actual observations in the field. In other words the surveyors map and describe soils as they find them in the field, that is, with varying amounts of moisture, depending on the seasonal conditions. In future work it seems probable that the same practice will be continued owing to the impossibility of mapping soils on an air-dry basis.

It is obvious, then, that one of the first steps necessary in the solution of the problem of soil color is the determination of the effects of varying moisture content on the color and particularly on the color descriptions of various soils. Later it will of course be necessary to work out better terms to use in describing soils and to adopt color charts which may be used in the field. But the moisture problem must be solved first.

[1] Member of the Committee on Soil Color Standards of the American Association of Soil Survey Workers.

It is quite generally recognized that moisture exerts a very important influence on soil color. Soils in an air-dry condition are known to be lighter than when wet, the depth of color increasing with increases in moisture, up to a certain point. It is believed also that dark colored soils show greater change in color with variations in moisture, than do light colored types. However, the real effect of moisture on soil color has not been determined and it is not known whether or not there is any definite relation between the two.

The purpose of the work reported in this paper was to study briefly and preliminarily the effect of varying moisture content on the actual color of two soil types which occur rather extensively in Iowa. The differences in color are shown in plate 1. The variations in descriptions, using the colors given by Ridgway (2) are shown in the tables.

TABLE 1

*Clinton silt loam*

| MOISTURE CONTENT | COLOR OF SURFACE SOIL (RIDGWAY'S COLORS) | COLOR OF SUBSOIL (RIDGWAY'S COLORS) |
|---|---|---|
| *per cent* | | |
| Air-dry | Avellaneous, *slightly yellow* | Warm buff |
| 10 | Tawney Olive, *slightly light* | Cinnamon buff |
| 20 | Saccardos Umber, *slightly light* | Tawney olive, *slightly light* |
| 30 | Saccardos Umber, *slightly dark* | Tawney olive |
| 40 | Saccardos Umber, *slightly dark* | Tawney olive |

TABLE 2

*Tama silt loam*

| MOISTURE CONTENT | COLOR OF SURFACE SOIL (RIDGWAY'S COLORS) | COLOR OF SUBSOIL (RIDGWAY'S COLORS) |
|---|---|---|
| *per cent* | | |
| Air-dry | Drab, *slightly dark* | Pinkish buff |
| 10 | Buffy Brown, *dark* | Clay color, *light* |
| 20 | Olive Brown, *dark* | Tawney olive, *dark* |
| 30 | Clove Brown, *light* | Saccardos umber |
| 40 | Clove Brown, *light* | Saccardos umber |

The soils studied were the Clinton silt loam, a light colored type and the Tama silt loam a dark colored soil. Both soils are of loessial origin, the former being formed under timbered conditions, while the latter has developed on the prairies. Samples of these soils were chosen which were as closely typical of the types as it is possible to secure.

The colors of the surface soil and of the subsoil samples were carefully noted under uniform light conditions. The colors were determined on the air-dry soils and on samples containing 10, 20, 30 and 40 per cent moisture. Considerable difficulty was experienced in selecting terms from Ridgway's colors which really expressed the color of the soils. There seems to be too wide an interval between the colors given by Ridgway to permit of definite description.

Thus the color of the surface soil of the Clinton silt loam in an air-dry condition was nearest *Avellaneous* but there was a slightly yellowish cast to the color. With 10 per cent moisture, the color of the soil was slightly light for Ridgway's *Tawney Olive*. In the tables therefore, Ridway's terms are modified to fit more closely the soil colors.

The reproduction of the various colors was accomplished with considerable difficulty by the use of oils and many attempts were made before the colors could be reproduced accurately. With experience, excellent results could undoubtedly be secured more readily.

Examining the results given in the tables and in the figures, it will be noted that with both soils, the color becomes darker as the moisture content increases until 30 per cent is reached. Beyond that point there is little change. It is interesting to note that the Clinton silt loam, with 20 per cent of moisture resembles closely the Tama silt loam in an air-dry condition. When it contains 30 per cent moisture it is very nearly the color of the Tama with 10 per cent. If this is true for soils which are typical, it can readily be conceived that there might be a greater overlapping when variations within the soils are considered. For example the darkest Clinton silt loam and the lightest Tama would overlap much further when the moisture content was different. Other soil types likewise might show marked similarity with different conditions of moisture whereas they would be quite different when in an air-dry condition.

Of course the field man makes some allowance for the ever-changing moisture factor but it is extremely doubtful if men can consistently do this from month to month and year to year with nothing more tangible to check against than their own individual interpretation of color descriptions.

The degree of color change between the soil in an air-dry condition and with 10, 20 and 30 per cent moisture is more pronounced in the case of the Tama silt loam than with the Clinton. This would seem to indicate that the darker colored soils or those richer in organic matter show greater variations in color with changing moisture conditions. The color change however is not uniform. Not only is the color darkened with more moisture but different shades and tints are brought out. This is more noticeable in the light colored soil.

It seems evident from this brief study that the moisture factor may play a most important part in determining the color of soils and in the descriptions which are given in the survey reports. The need for a definite understanding of the significance of the moisture factor in field soils when they are being mapped is obvious. The field men should probably be provided with color charts showing the color of the typical soils with varying moisture content, 10, 20 and 30 per cent, and also air-dry. In this way the personal equation factor would operate only to the extent that the surveyor would need to judge of the the moisture content. This he could undoubtedly do quite accurately and certainly he would be able to fix the soil color much more definitely than if he is obliged to guess at the probable color of the soil, if the moisture content were

lower or higher, as it might have been a day or two previous. Perhaps with further study, it may be possible to fix on definite colors with 10 and 30 per cent moisture, the intermediate content not being of sufficient effect to warrant consideration. If this is possible, the problem will be much simplified for these amounts would be easily translated by the field man into "dry" and "moist" and he could readily make a differentiation. Further work is of course necessary before such a solution of the problem of moisture and soil-color is possible. The effects of smaller differences in moisture than those used here must be tested. A wide variety of soils must be examined as the types used in this work may not indicate the results which may be secured with other light colored and dark colored soils. Further, more comprehensive study of moisture effects on soil color is certainly very desirable.

The adoption of more definite terms for describing soil color is also quite evidently necessary. Ridgway's color standards are good as far as they go but the differences between his colors are too great. Then, too, the terms which he uses are cumbersome, meaningless and useless from the practical standpoint. The color names selected by the Color Standards Committee of the American Association of Soil Survey Workers are much better but they are not nearly complete enough to be satisfactory. It is hoped that further work by this committee may lead to the adoption of a complete and reasonably simple method of describing soil colors.

Definite conclusions of course cannot be drawn from the meager data presented here but it is hoped that the report may serve to stimulate further study along this line and to hasten the time when soil colors may be more definitely described and the field men may experience less difficulty in mapping soils and in describing them.

## REFERENCES

(1) BROWN, P. E., AND O'NEAL, A. M. 1923 The color of soils in relation to organic matter content. Iowa Agr. Exp. Sta. Res. Bul. 75.

(2) RIDGWAY, R. 1912 Color Standards and Color Nomenclature. Mrs. J. E. Ridgway, Olney, Ill.

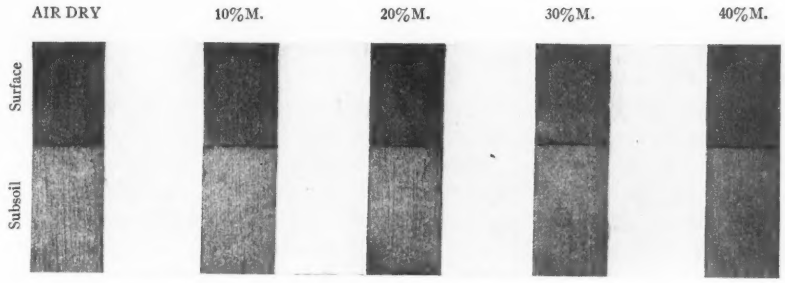


FIG. 1. CLINTON SILT LOAM

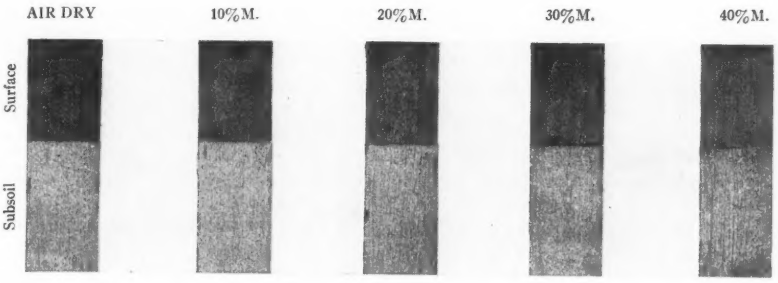


FIG. 2. TAMA SILT LOAM

# ORGANIC PHOSPHORUS OF SOILS[1]

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The amounts of organic phosphorus in soils are so considerable that their significance and possible application to practical agriculture cannot be overlooked. When 30 to 40 per cent of the total soil phosphorus is found in organic combination, its importance becomes apparent (3). The compounds in which phosphorus is combined in plants are quite well known and have been the starting point for several theories as to their final transformation; however, aside from the isolation of nucleic acid by Shorey (19) and lecithin by Aso (2), practically nothing has been done in the way of their identification in soils.

Very large amounts of organic phosphorus must be added to soils each year from plant growth. Nucleic acid is an important constitutent of every living cell. Maclean (12) states that the phosphatides appear to be present in every animal and vegetable cell so far investigated. However, Plimmer (15) in classifying the organic phosphorus compounds says "Phospholipins of which lecithin is the principal one, occur chiefly in animals and only to a small extent in plants." Aso (2) in summing up his work on organic phosphorus compounds in soils says "The quantity of lecithin is small so it cannot be taken into serious consideration." So far phytin has not been isolated from soils. Quite large amounts of this compound occur in seeds. Suzuki and Yoshimura (22) found 85 per cent of the phosphorus in clover to be phytin. Briefly then quite large amounts of nucleic acid and phytin and relatively small amounts of phosphatides are added to soils.

Gortner and Shaw (8) suggested that part, if not all, of the organic phosphorus compounds found in soils was extracted from living bacteria or protozoa.

Waksman (23), (24) found the highest bacterial number of any time of the year was 21,700,000 per gram of soil; ciliates and amoeba, 10 to 100 per gram; and flagellates 1,000 to 10,000 per gram. Waksman and Curtis (25) found the average number of actinomyces to be 933,000 per gram. Calculating the weight of all the bacteria in the surface soil of an acre according to the figures given by Marshall (14) and using 1.3 per cent phosphorus as an average on the dry weight, only approximately .09 pounds of phosphorus can be accounted for. Using Marshall's figures again, and calculating the volume of organisms necessary to represent 300 pounds of organic phosphorus, there would be required in the surface alone a solid layer of living organisms 2.64 inches deep over the entire acre. Carrying the *reductio ad absurdum* still farther to include all organisms, assuming a 20 per cent dry matter content and a 1.3 per cent phosphorus content, there would be required for the 1566.22 pounds of organic phosphorus in all three depths of soil no. 4 (3) a solid layer of living organisms over 3 inches thick covering

[1] The author feels indebted to Dr. P. E. Brown for helpful suggestions in correcting and editing this manuscript; also to Dr. Paul Emerson and Dr. H. W. Johnson for many kind and pertinent suggestions during the carrying out of this work.

the entire acre. The latter allows for no space between organisms. If this be taken into account, the figures would necessarily be greater. Some large amounts of organic phosphorus are found in poor soils where the bacterial count would be low and in subsoils where it is generally conceded that living organisms are few. There must be some other source of organic phosphorus than living organisms.

Moreover, the ease of hydrolysis of nucleic acid, phytin, and lecithin would seem to eliminate them from consideration as making up any considerable portion of the large amount of organic phosphorus found in some cases. Nucleic acid is easily hydrolyzed by certain organisms (10). Lecithin is easily broken down by acids or bases, and phytin is quickly hydrolyzed by phytase (1). Then, too, nucleic acid, phytin, and lecithin are readily available to plants (20), which fact further removes them from consideration as a possible form of accumulated unavailable phosphorus.

It has been a rather generally accepted fact that soil organisms serve as a medium for storage of plant food. In reality it is doubtful if they are very important in this way. Even if their entire mass contained enough plant food to be of consequence it would be in the living organism and unavailable at the time when the plant most needed it.

It has been suggested by Potter and Benton (16) that perhaps the accumulation of organic phosphorus was due to pyrimidine nucleotides. This theory originated because of the similarity of the curves of hydrolysis of nucleic acid and ammonia extracts of soil. The theory was further substantiated by Schollenberger (18). No attempt has been made to determine nucleotides or nucleic acid in soil quantitatively. Funatsu (7) incubated nucleoprotein from beer yeast in sand cultures and found only 39 per cent of the phosphorus hydrolyzed in two months. The fact that nucleic acid was found in soils speaks well for the technique of the investigators and was a splendid piece of work, but in view of the very small amounts of nucleic acid or of its hydrolytic products, the purines and pyrimidines and in consideration of facts to appear later in this paper, there seems to be good evidence to indicate that nucleic acid does not accumulate in soils, either as such or as the pyrimidine half of the molecule.

Although Fraps (6) rendered untenable the assertion of Stewart (21) that the increase in soluble phosphorus after heating is organic and quantitative it seems that, although perhaps not absolutely quantitative, Schmoeger's (17) idea that heating liberated phosphorus from organic combination, has good foundation.

Briefly summing up the generally accepted facts based on work done up to this time:

1. Organic phosphorus in considerable amounts exists in soils.
2. An apparently satisfactory method for estimating organic phosphorus in ammonia extracts has been developed.
3. Phosphorus exists in soils as phospholipins, nucleic acid, and considering the amounts added probably as some phytin; perhaps, also, as an accumulation of pyrimidine nucleotides.

## EXPERIMENTAL

The following experiments were carried out with the idea of making a critical study, first, of the method of Potter and Benton; second, to determine whether organic phosphorus in soils is in chemical combination or adsorbed; third, to discover if possible the nature of the organic phosphorus compounds.

The soil selected was Jackson silt loam. This particular soil was selected because of its high content of organic phosphorus; then, too, because of its low organic matter content it gave a more easily filterable ammonia extract. The soil is described as follows: "The surface soil is a light-brown, smooth, silt loam to a depth of 8–12 inches and passes into a yellowish-brown silty clay loam to silty clay. The subsoil is rather compact." The application of lime where the soil is acid and of phosphorus and organic matter gives good results.

In this section of the experimental work 200 gm. of soil were shaken for one hour with 1 per cent hydrochloric acid, filtered, washed, dried, reground, and shaken for 8 hours with 500 cc. of 4 per cent ammonia. The soil was separated from the extract by means of a separator running at a speed of 20,000–25,000 R.P.M. The method of determining organic phosphorus on this extract was that used in the previous experiments (3).

It has been noted that phosphorus is separated from guanine with some difficulty when isolating the purines or nucleic acid. It was thought possible that the purines or pyrimidines, existing in soils as a result of the hydrolysis of nucleic acid, might be dissolved in the ammonia extract and, when magnesia mixture was added, form, with the organic matter, a complex magnesium purine or pyrimidine phosphate. The inorganic phosphorus would thus be removed from solution in a form which would not be redissolved by dilute nitric acid and in consequence be calculated as organic. In order to test this supposition, various amounts of the purine and pyrimidines (isolated from yeast nucleic acid) were added to 100 cc. portions of the ammonia extract and the usual procedure for determining organic phosphorus carried out. The precipitates from magnesia mixture after having been washed with dilute nitric acid were analyzed for total phoshorus by the usual magnesium nitrate method. Such a voluminous precipitate was secured and it was of such a nature that although the washing with nitric acid was thorough, some inorganic phosphorus undoubtedly was left in an adsorbed state. The results secured can be considered only as comparative. The experiment was repeated using only guanine and xanthine. Concordant results were secured; but there was not enough difference to warrant the assumption that an organic phosphorus compound was formed during the precipitation with magnesia mixture. In fact in the first experiment the purines seemed to increase the magnesia precipitate while in the second the guanine seemed to increase the amount of inorganic phosphorus.

Table 1 shows the original experiment; table 2 the amino purine and oxypurine test. It might well be stated that the results for phosphorus here as well as elsewere in this paper are given in their equivalent in cubic centimeters of 0.1 *N* alkali. The total phosphorus on 100 cc. of extract was equivalent to 53.4 cc. of 0.1 *N* alkali. The 25 cc. of phosphate solution was equivalent to 24.4 cc. of 0.1 *N* alkali. In table 5, 10 cc. of phosphate solution equivalent to 9.76 cc. of 0.1 *N* alkali were used. The purine and pyrimidines were prepared from yeast nucleic acid according to the Jones method (11). In the removal of picric acid from adenine it was found that a more satisfactory separation than the ether-acid procedure was secured by dissolving the adenine picrate in ammonia, precipitating it with silver nitrate, washing and then boiling the well washed adenine silver salt with hydrochloric acid. The filtrate contained a known amount of the chloride and was diluted to the required volume. Choline was prepared from egg lecithin by hydrolysis with baryta, precipitation with platinum chloride, and subsequent separation with hydrogen sulfide.

TABLE 1

*Effect of various amounts of organic bases on the phosphorus content of the washed magnesia mixture precipitate*

| BASE ADDED TO 100 CC. SOIL EXTRACT PLUS 25 CC. PHOSPHATE SOLUTION* | | PHOSPHORUS AS 0.1 *N* ALKALINE EQUIVALENT |
|---|---|---|
| Kind | Amount | |
| | *mgm.* | *cc.* |
| Adenine | 75 | 41.8 |
| | 50 | 39.7 |
| | 5 | 38.7 |
| Guanine | 100 | 40.4 |
| | 50 | 37.1 |
| | 5 | 39.9 |
| Uracil | 100 | 40.6 |
| | 50 | 42.1 |
| | 5 | 43.1 |
| Cytosine | 100 | 42.6 |
| | 50 | 44.1 |
| | 5 | 43.0 |
| Choline | 100 | 43.5 |
| | 50 | 43.8 |
| | 5 | 46.7 |
| Xanthine | 100 | 49.0 |
| | 25 | 45.9 |
| | 5 | 45.1 |
| Hypoxanthine | 100 | 43.5 |
| | 50 | 47.0 |
| | 5 | 44.0 |
| None | | 46.4 |

* 25 cc. standard phosphate solution was equivalent to 24.4 cc. 0.1*N* alkali.

TABLE 2

*Phosphorus in $HNO_3$ extract of magnesia mixture precipitate*

| SOIL EXTRACT | BASE ADDED | | PHOSPHATE SOLUTION ADDED* | PHOSPHATE EQUIVALENT IN 0.1 *N* ALKALI |
|---|---|---|---|---|
| | Kind | Amount | | |
| *cc.* | | *mgm.* | *cc.* | *cc.* |
| 100 | Guanine | 50 | 10 | 21.3 |
| 100 | Xanthine | 50 | 10 | 19.4 |
| 100 | None | None | | 10.2 |

* 10 cc. standard phosphate solution was equivalent to 9.76 cc. 0.1*N* alkali.

The experiment shows that it is unlikely that the purines or pyrimidines, even if present in the soil in large amounts, would influence the determination of organic phosphorus.

In order to determine whether phosphorus was held in chemical combination in the ammonia extract or was simply adsorbed by colloids, equal amounts of the soil extract were treated with varying amounts of standard phosphate solution. The dilute nitric acid filtrate, the washed residue and the first filtrate from the magnesia precipitation were analyzed separately for phosphorus. Table 3 gives the results. It may be seen that complete recovery was made of the phosphorus added. There was no increase in the phosphorus in the residues and the first filtrate showed a constant amount. It must be concluded that appreciable adsorption did not take place or complete recovery of the added phosphorus would not have resulted. Because of the increased concentration of phosphorus there would have been a difference between the 25 cc. application and the 50 cc. application.

TABLE 3

*Effect of adding phosphorus in different concentrations*

| SOIL EXTRACT* | STANDARD PHOSPHATE SOLUTION ADDED† | PHOSPHORUS EQUIVALENT IN CC. 0.1 *N* ALKALI | | |
|---|---|---|---|---|
| | | $HNO_3$ filtrate | Washed residue | First filtrate |
| *cc.* | *cc.* | *cc.* | *gm.* | *cc.* |
| 50 | 25 | 29.4 | 14.7 | 6.7 |
| 50 | 50 | 53.9 | 14.8 | 6.7 |

* 50 cc. soil extract contains 5.1 cc. phosphorus equivalent in 0.1*N* alkali.

† 25 cc. standard phosphorus contains 24.4 cc. phosphorus equivalent in 0.1*N* alkali. 50 cc. standard phosphorus contains 48.8 cc. phosphorus equivalent in 0.1*N* alkali.

There is another possibility in connection with the precipitation of inorganic phosphorus from ammonia extracts and that is the difference in precipitating agents. It was decided to try out varying amounts of magnesia mixture in order to determine if the mass action of an increased magnesium ammonium content would increase the amount of inorganic phosphorus precipitated. Saturated solutions of ammonium chloride and ammonium nitrate were also used as precipitants with interesting results.

Table 4 shows the results secured with different amounts of magnesia mixture and with saturated solutions of ammonium chloride and ammonium nitrate. Figures for the inorganic phosphorus in the dilute nitric acid filtrate as well as for phosphorus on the washed magnesia mixture precipitate and on the first filtrate from the magnesia mixture precipitate are given. In column 3 are the figures for the amounts of inorganic phosphorus found in the filtrates from ammonium chloride and ammonium nitrate precipitations. Table 4 shows that the amount of magnesia mixture has very little effect in increasing the amount of inorganic phosphorus. In the case of the 10 cc. addition, the small amount affected the distribution between the nitric acid insoluble organic and the soluble organic in the filtrate. The inorganic phosphorus con-

tent was not different from that secured by the addition of the large amounts. Very interesting results may be observed in the case of the ammonium salts. Here practically all of the inorganic phosphorus has been salted out along with the organic compounds. Very different results were anticipated; namely, that very little inorganic phosphorus would have been precipitated and much organic; but just the opposite occurred. Almost complete precipitation of inorganic phosphorus was accomplished. The phosphorus which remained after filtration from the ammonium salts was equivalent to only 0.3 cc. 0.1 *N* alkali, in the case of ammonium chloride, and 0.55 cc. in the case of ammonium nitrate. The surprising thing about this precipitation with ammonium salts was the large amount of organic phosphorus left in solution as compared with that left in the case of magnesia mixture (a 22-cc. equivalent against a 6-cc. equivalent in the case of the extract precipitated with 50 cc. of magnesia mixture), nearly four times as much. Evidently magnesia mixture is a precipitant of the organic compounds but it failed to precipitate any of the large

TABLE 4

*Effect of different precipitants in varying amounts*

| PRECIPITANT | PHOSPHORUS GIVEN IN TERMS OF 0.1 *N* ALKALI | | | |
|---|---|---|---|---|
| | Inorganic phosphorus | Phosphorus in first filtrate | Inorganic phosphorus in filtrate from ammonium salts | Phosphorus in residues |
| | *cc.* | *cc.* | *cc.* | *cc.* |
| 10 cc. magnesia mixture | 9.65 | 8.35 | | 35.4 |
| 25 cc. magnesia mixture | 9.30 | 6.4 | | 37.7 |
| 50 cc. magnesia mixture | 9.9 | 6.0 | | 37.5 |
| 100 cc. magnesia mixture | 9.1 | 6.8 | | 37.3 |
| Saturated $NH_4Cl$ | 8.45 | 22.0 | 0.3 | 22.95 |
| Saturated $NH_4NO_3$ | 8.95 | 22.0 | 0.55 | 22.45 |

remaining amount after the salting out with ammonium salts. These facts prove quite conclusively that the phosphorus in ammonia extracts of soil is largely organic for were it inorganic it would have been precipitated by magnesia mixture even though large amounts of ammonia salts were present. The removal of so much troublesome organic material by salting out with ammonium salts, leaving so large a proportion of the organic phosphorus in the filtrate suggests a possible means of isolation of some of the organic phosphorus compounds; namely, dialysis of the ammonium salts and crystallization following evaporation and solution in suitable solvents.

There seems to be no tendency for large amounts of magnesia mixture to increase the amounts of inorganic phosphorus. The variation in individual tests is so large that further work on a large number of samples will be necessary in order to settle this point. Any difference, however, is probably small.

The precipitate formed by adding magnesia mixture to ammonia extracts is so voluminous in some cases and of such a nature that the possibility of its

adsorbing and enclosing phosphorus between its finely divided particles is worthy of consideration. One hundred cubic centimetres of ammonia extract was precipitated with magnesia mixture and the precipitate thoroughly washed with dilute nitric acid as in the regular method. The residue on the paper was shaken up with dilute nitric acid in an Erlenmeyer flask and again filtered and washed. This residue was then allowed to stand covered with 100 cc. of distilled water for 24 hours. At the end of this time it was filtered and inorganic phosphorus very carefully determined on the filtrate. The residue was again allowed to stand with 100 cc. of distilled water for 10 days; then for six weeks. The inorganic phosphorus which diffused out is shown in table 5.

It may be seen that in the case of sample 1 an amount of inorganic phosphorus equivalent to 4.5 cc. 0.1 *N* alkali was diffused out of the precipitate. This of course in the regular procedure would be counted as organic. There is the possibility of this phosphorus having been hydrolyzed from the organic phosphorus compounds, inasmuch as the precipitate enclosed some nitric acid from the dilute nitric acid wash. The acid concentration in the second and

TABLE 5

*Inorganic phosphorus diffused from washed residues*

| SAMPLE | 0.1 *N* ALKALI EQUIVALENT TO PHOSPHORUS DIFFUSED | | | |
|---|---|---|---|---|
| | First period (48 hours) | Second period (10 days) | Third period (6 weeks) | Total |
| | *cc.* | *cc.* | *cc.* | *cc.* |
| 1 | 1.9 | 1.4 | 1.2 | 4.5 |
| 2 | 1.9 | 1.7 | 1.1 | 4.7 |
| 3 | 1.8 | 1.3 | 0.0 | 3.1 |

third periods, however, was very low. From an analytical point of view the presence of this phosphorus indicates the necessity of using an ammonia extract representing as small an amount of soil as is practical thus avoiding the voluminous precipitate. This inorganic phosphorus recovered represents 12 per cent of the total contained in the washed magnesia mixture precipitate. If it is hydrolyzed from organic compounds it would not necessarily indicate an ease of hydrolysis of such compounds in soil inasmuch as the precipitation and various washings have changed its nature and probably freed it from retarding enzymatic factors. Old infertile soils sometimes have high ratios of organic to inorganic phosphorus which indicate a low availability of organic phosphorus. The probabilities are that the 12 per cent recovered was largely inorganic adsorbed or enclosed.

When the possibilities of organic phosphorus being in soils as nucleic acid, phytin, and lecithin are considered, the question immediately arises as to the rates of hydrolysis of these compounds. Are they able to withstand bacterial activities? In the case of nucleic acid is the pyrimidine half of the molecule resistant to enzymes? In order to settle this point, nucleic acid, phytin, and

lecithin were incubated in silica sand with soil infusion. The sand had previously been digested for several days with hydrochloric acid, washed free of acid, and dried. By analysis it showed 99 per cent $SiO_2$.

Table 6 shows the results. One hundred milligrams of nucleic acid, 100 mgm. of phytin, and an amount of lecithin in absolute ether equivalent to 79.2 cc. 0.1 *N* alkali were mixed with 100 gm. of silica sand. The nucleic acid contained no inorganic phosphorus precipitable with magnesia mixture. The 100 mgm. phytin contained phosphorus equivalent to 3.3 cc. 0.1 *N* alkali. The lecithin contained in 20 cc. of the ether solution, inorganic phosphorus equivalent to 12 cc. 0.1 *N* alkali. The inoculated silica sand cultures were allowed to incubate at room temperatures and the inorganic phosphorus determined at one month intervals.

The method used for determining inorganic phosphorus in nucleic acid cultures consisted in adding ammonia and 25 cc. of magnesia mixture and after standing over night filtering off the unchanged nucleic acid. The residue was thoroughly washed with water containing ammonia; then the inorganic magnesium ammonium phosphate was dissolved out with dilute nitric acid, the

TABLE 6

*Hydrolysis of organic phosphorus compounds*

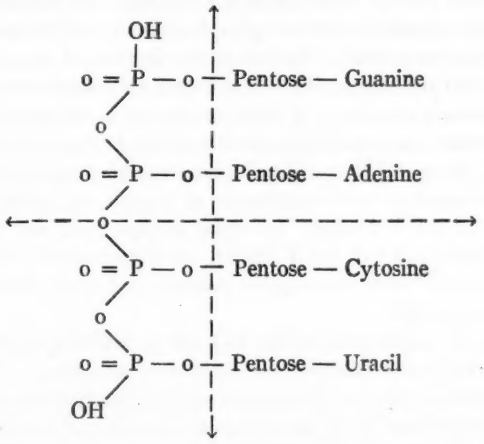
| COMPOUND | AMOUNT HYDROLYZED AT END OF | | |
|---|---|---|---|
| | First month | Second month | Third month |
| | *per cent* | *per cent* | *per cent* |
| Nucleic acid | 80.7 | 85.6 | 85.04 |
| Phytin | 39.3 | 64.9 | 67.00 |
| Lecithin | 56.9 | 59.8 | 66.00 |

solution thoroughly oxidized and the phosphorus determined in the usual way by molybdate precipitation. Phytin was determined by the acid alcohol method of Forbes (5). It was necessary to work out a method for lecithin, and this was done as follows. Lecithin is insoluble in acetone. By using 10 cc. of nitric acid (sp. gr. 1.42) per liter of acetone, a solvent was found which proved very successful in dissolving the inorganic phosphorus and any glycerophosphoric acid. The mixtures of glycerophosphoric acid and phosphoric acid were taken as a measure of the hydrolytic power of the soil. The method was tested out as follows. Commerical lecithin was dissolved in alcohol, precipitate with acetone, dried and dissolved in absolute ether. Five cubic centimetres of this solution were added to about 50 gm. of silica sand and allowed to evaporate. The dried silica sand was then extracted with the acid acetone. An amount of inorganic phosphorus equivalent to 3 cc. of 0.1 *N* NaOH was obtained in the filtrate. Another 5-cc. portion of lecithin solution in sand was extracted with pure acetone and the extract evaporated. The residue was oxidized with nitric acid and the resulting solution after dilution and filtration tested for phosphorus in the usual way. An amount of phosphorus

equivalent to 3 cc. 0.1 *N* NaOH was secured. This showed that the short extraction with acid acetone did not hydrolyze any appreciable amount of the lecithin. The small amount of inorganic phosphorus found in each case was evidently left from the purification process or hydrolyzed subsequently. In order to test the solubility of inorganic phosphorus in acid acetone, 25 cc. of standard phosphate solution, equivalent to 21.1 cc. 0.1 *N* NaOH was evaporated to dryness and mixed with silica sand. Five cubic centimeters of lecithin solution in ether was next added and allowed to evaporate. Extraction of this sand mixture with acid acetone showed a complete recovery of both the inorganic phosphorus in the lecithin equivalent to 3 cc. 0.1 *N* NaOH and that in the standard equivalent to 21.1 cc. 0.1 *N* NaOH.

Table 6 shows that at the end of three months 85.04 per cent of the phosphorus of nucleic acid has been changed to the inorganic form, 67 per cent of the phytin phosphorus, and 66 per cent of the lecithin phosphorus.

Plant nucleic acid written structurally is shown below.




The horizontal dotted line divides the molecule into purine and pyrimidine nucleotides. The vertical line shows how phosphorus splits off on hydrolysis giving phosphoric acid and nucleosides. The latter process is probably what occurs in soils. The theory that pyrimidine nucleotides remain as such in soils cannot be a general truth for in the case of the incubation mentioned at least 70 per cent of the pyrimidine phosphorus has been split off from the molecule. In the case of phytin and lecithin the hydrolysis proceeded rapidly and probably in soils under conditions of drainage and aeration would rapidly approach completion. No doubt there are always small amounts of all three compounds in most soils. Nucleic acid has unquestionably been isolated as has lecithin, though in small amounts. Phytin has so far not been isolated. None of these compounds or their hydrolytic products are difficult to isolate

if present in large quantities. Were 300 pounds of phosphorus to be calculated to either of the pyrimidine nucleotides, there would be about 3000 pounds of cytosine or uracil nucleotide in an acre representing 1000 pounds of either cytosine or uracil. Were the bases in equal amounts, there would be 500 pounds of each in the surface soil of an acre, an amount which could not fail of detection.

Twenty kilograms of Jackson silt loam were extracted with ammonia following HCl extraction and the ammonia extract hydrolyzed with sulfuric acid. The resulting hydrolyzed mixture was treated for the isolation of purines and pyrimidines. The characteristic silver purine and pyrimidine precipitates were noted, but the amounts were so small as to render isolation and identification impossible. Hypoxanthine was isolated in very small amounts from the hydrochloric acid extract.

Maillard (13) in 1912 found that carbohydrates and amino acids may be condensed to form a humus-like substance. This artificial humus is soluble in ammonia and may be reprecipitated by acids. The following experiment was conducted to ascertain whether phosphorus, too, could be made to combine in the condensation product. Sixteen grams dextrose, 4 gm. alanine, 0.5 gm. $Na_2HPO_4 \cdot 12\ H_2O$ and 16 gm. water were heated for 3 hours in a steam pressure oven at 25 pounds pressure. A dark brown brittle substance was obtained which was treated for organic phosphorus by the Potter and Benton method. The ammonia extract of this artificial humus was black and resembled the soil extract. One hundred cubic centimeters of it gave total phosphorus equivalent to 14.9 cc. 0.1 *N* NaOH. An equal aliquot contained inorganic phosphorus equivalent to 4.5 cc. 0.1 *N* NaOH. A large amount of the phosphorus had been condensed with the complex molecule and could not be precipitated with magnesia mixture.

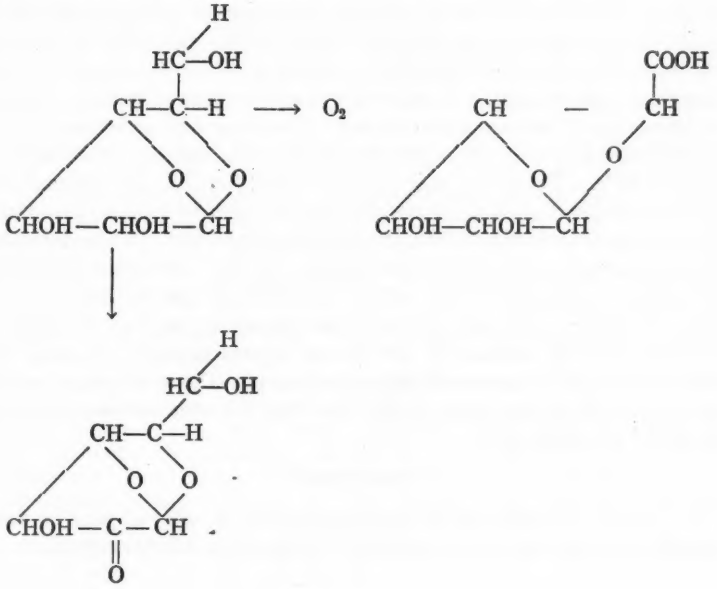
Dextrose and alanine refluxed for 15 hours at 100°C. gave the same black residue to which in ammonia solution phosphorus was added. Upon the addition of magnesia mixture the phosphorus was completely recovered. One hundred cubic centimetres of the same ammonia solution of artificial humus containing 25 cc. of standard phosphate solution was acidified with hydrochloric acid; then made alkaline with ammonia and precipitated with magnesia mixture. The same was repeated except that the standard phosphate solution was added after acidifying. In both cases complete recovery of phosphorus resulted. These tests were made to ascertain whether the phosphorus was absorbed from the acid solution as would be the condition in the preliminary acid extraction. Very probably the organic phosphorus is present in soils as the calcium, magnesium, iron, or aluminum salts of the phosphorus, carbohydrate, amino acid complex and not absorbed from the phosphoric acid liberated by the hydrochloric acid from inorganic phosphorus salts. There are two possibilities at least, (a) that the organic phosphorus is formed under conditions existing when the hydrochloric acid liberates inorganic phosphorus from inorganic salts or (b) that it exists as the calcium salt of the organic phosphorus

complex which when the calcium is removed becomes soluble because of the formation of the ammonium salt. It is a well known fact that without previous acid extraction ammonia will dissolve very little organic phosphoric acid; but after HCl extraction practically all of it is dissolved. It seems very reasonable to infer that the organic phosphorus exists as organic in the soil and is not formed under the conditions of the experiments.

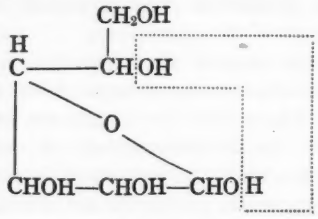
Another interesting observation was made of the artificial humus. Since the condensation between dextrose and alanine went on rather slowly at 100°C., tests were made from time to time. The product first formed was readily soluble in ammonia as well as in acids. As the condensation went on and the product became more and more complex, while it remained soluble in ammonia, it became insoluble in acids. At the end of the process the mixture might have been divided into three parts: ammonia insoluble, ammonia soluble but acid insoluble, and ammonia and acid soluble. The ammonia insoluble corresponds to humin, the ammonia soluble acid insoluble to humus, and the red acid soluble portion to Mulders apocrenic acid.

Beckley (4) has recently written a very interesting article on the formation of humus. He points out that cellulose broken down into its components is changed to hydroxymethyl furfural and this in turn condenses to form humus.

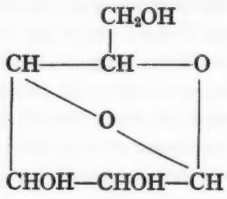
Hibbert (9) suggests a formula for cellulose and explains why oxycelluloses are either alkali soluble or alkali insoluble. His formulae below show how by oxidation the carboxy- or alkali-soluble oxycellulose is formed in one case and the ketonic or alkali-insoluble form in the other.



Hibbert also explains the process of changing cellulose to hydroxymethyl furfural. Cellulose hydrolyzes quantitatively to give dextrose. The relationship between dextrose and the cellulose nucleus Hibbert graphically represents as below.

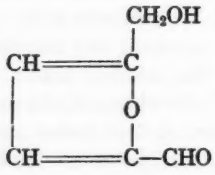



*dextrose*




*cellulose nucleus*

By heating, cellulose is partially converted into w hydroxymethyl furfural




According to Beckley (4) humus is formed by polymerization or condensation of this compound. We can then easily follow the formation of humus from cellulose. It is not difficult to conceive of a complex condensation product which has both hydroxyl groups and carboxyl groups and which might easily condense or combine with phosphoric, sulfuric or other acids and at the same time form a calcium salt. It seems very likely that humus is such a complex. As humus ages it becomes more complex. Phosphoric acid and other elements become buried, so to speak, in the complex molecule and as a consequence are less available. The liberation of phosphoric acid from the carbonaceous complex is probably dependent for the most part in the older humus to oxidation. In the younger formations no doubt hydrolysis plays a part. These deductions throw some light on the organic phosphorus situation. Phosphorus locked up in organic combination in old soils is less available and remains while the inorganic phosphorous and more available organic are used up by plants or leached; hence the increase in the ratio of organic to total. We may, too, explain the hydrolysis curve of ammonia extracts. The more simple complex gives up its phosphorus more readily than does the more complex; hence the similarity to nucleic acid.

## CONCLUSIONS

1. Organic phosphorus exists in large amounts in soils and may be determined with a fair degree of accuracy by the Potter and Benton method.

2. Organic phosphorus probably does not exist in any considerable amount as nucleic acid, phytin, or lecithin.

3. Organic phosphorus in soils is probably not an accumulation of pyrimidine nucleotides.

4. Organic phosphorus compounds added to soils are probably hydrolyzed and the inorganic phosphorus combined in a calcium-magnesium or other metal salt of an organic amphoteric complex.

## REFERENCES


(1) ANDERSON, R. J. 1915 The hydrolysis of phytin by the enzyme phytase contained in wheat bran. *In* Jour. Bio. Chem., v. 20, 475.

(2) ASO, K. 1904 On organic compounds of phosphorus in soils. *In* Bul. Col. Agr. Tokio Imp. Univ. (Japan), 6, p. 277–284.

(3) AUTEN, J. T. 1922 The organic phosphorus content of some Iowa soils. *In* Soil Sci., v. 13; p. 119–124.

(4) BECKLEY, V. A. 1921 The formation of humus. *In* Jour. Agr. Sci., v. 11, p. 69–77.

(5) FORBES, E. B., LEHMANN, A., COLLISON, R. C., WHITTIER, A. C. 1910 Methods for the quantitative estimation of inorganic phosphorus in vegetable and animal substances. Ohio Agr. Exp. Sta. Bul. 215.

(6) FRAPS, G. S 1911 Organic phosphorus of soils. Tex. Agr. Exp. Sta. Bul. 136.

(7) FUNATSU, T. 1908 On different forms of phosphoric acid in press Cake. *In* Bul. of Col. of Agr. Tokio Imp. Univ. (Japan), v. 7; p. 457–459.

(8) GORTNER, R. A., AND SHAW, W. M. 1916 The organic matter of the soil. IV. Some data on humus phosphoric acid. *In* Soil Sci., v. 3, p. 99.

(9) HIBBERT, H. 1921 The constitution of cellulose. *In* Jour. Indus. and Engin. Chem., v. 13, no. 4, p. 334–342.

(10) IWANOFF, L. 1903 Ueber die fermentative Zersetzung der Thymo-nuclinsaure durch Schimmelpilze. *In* Zeitschr. Physiol. Chem., v. 39; p. 31.

(11) JONES, W. 1914 Nucleic Acids, Their Chemical Properties and Physiological Conduct. *Monograph*, Longmans, Green & Co., New York.

(12) MACLEAN, H. 1918 Lecithin and Allied Substances; The Lipins. *Monograph*, Longmans, Green and Co., N. Y.

(13) MAILLARD, L. C. 1912 Formation d'humus et de combustible mineraux sans intervention de l'oxygene atmospherique, des micro-organisms, des hautes temperatures, ou des fortes pressions. *In* Compt. Rend. Acad. Sci. (Paris), v. 155, p. 1554–1556.

(14) MARSHALL, C. E. 1912 Microbiology, p. 89. P. Blakiston's Sons and Co., Phila.

(15) PLIMMER, R. H. A. 1912 The Metabolism of Organic Phosphorus Compounds. Their Hydrolysis by Action of Enzymes. *Monograph*, Longmans, Green and Co., N. Y.

(16) POTTER, R. S., AND BENTON, T. H. 1916 The organic phosphorus of soils. *In* Soil Sci., v. 2, p. 291–298.

(17) SCHMOEGER, M. 1893 Ueber den Phosphor in Moorboden. *In* Ber. Deut. Chem. Gesel., Jahrg. 26, v. 1, p. 386.

(18) SCHOLLENBERGER, C. J. 1918 Organic phosphorus of soils: Work on methods for extraction and determination. *In* Soil Sci., v. 6, p. 365–395.

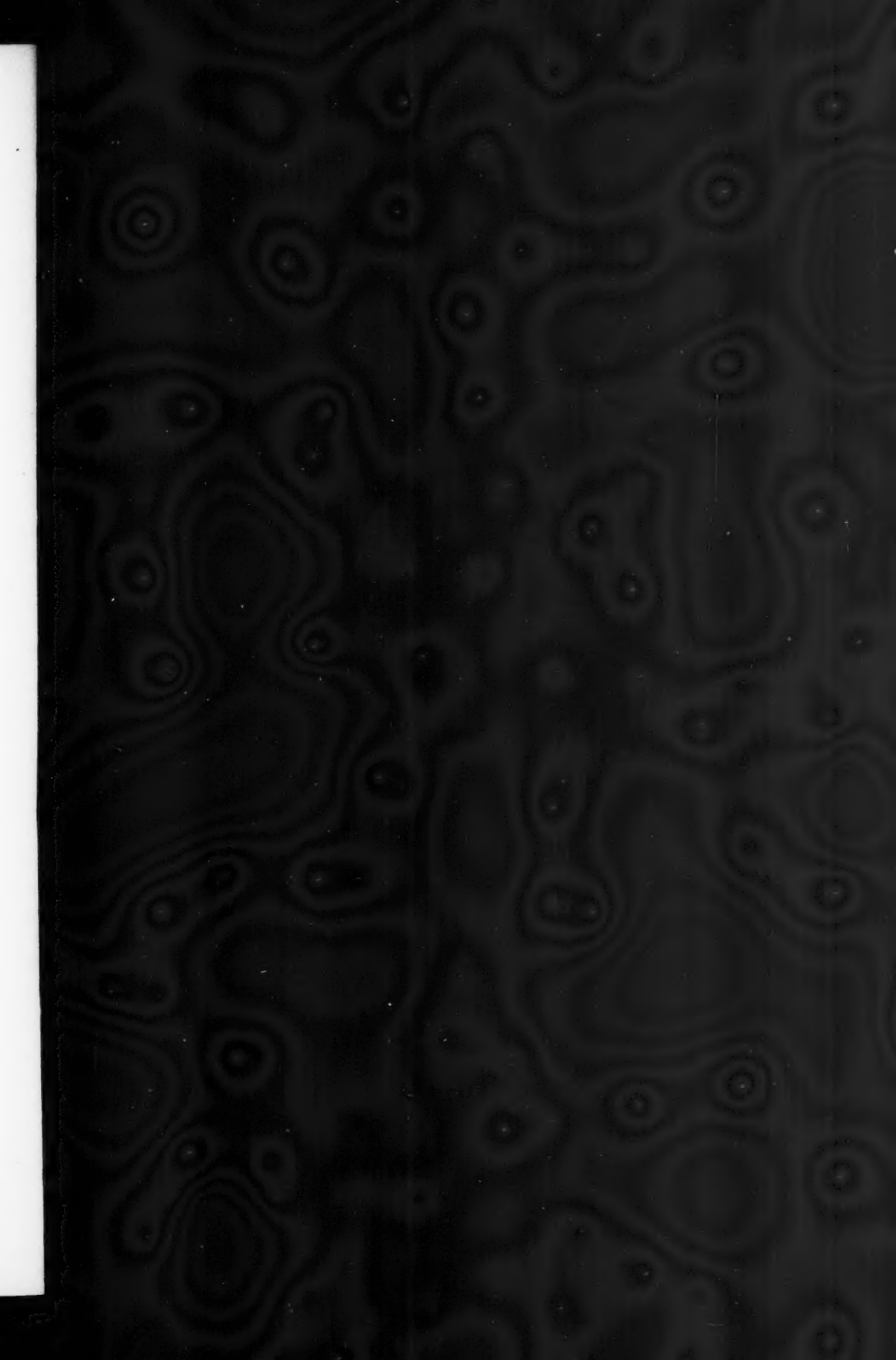
(19) SHOREY, E. 1911 Nucleic acid in soils. *In* Science, n. s., v. 35, p. 390.

(20) SCHREINER, O., AND SKINNER, J. J. 1912 Nitrogenous soil constituents and their bearing on soil fertility. U. S. Dept. Agr. Bur. Soils Bul. 87.

(21) STEWART, ROBT. 1910 Quantitative relationship of carbon, phosphorus, and nitrogen in soils. Ill. Agr. Exp. Sta. Bul. 145.

(22) SUZUKI, U., AND YOSHIMURA, K. 1907 Ueber die Verbreitung von Anhydro-Oxy-Methylen-Diphosphor Sauren oder "Phytin" in Pflanzen. *In* Bul. Col. Agr. Tokio Imp. Univ. (Japan), v. 7, p. 495–502.

(23) WAKSMAN; S. A. 1916 Bacterial numbers in soils at different seasons of the year. *In* Soil Sci., v. 1, p. 363.

(24) WAKSMAN, S. A. 1915 Studies on soil protozoa. *In* Soil Sci., v. 1, p. 140.

(25) WAKSMAN, S. A., AND CURTIS, R. E. 1916 The actinomyces of the soil. *In* Soil Sci., v. 1, p. 99.